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Biocatalysts in Cancer Tissue

III. Succinic Dehydrogenase and Cytochrome Oxidase*

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The accumulation of lactic acid in tumor tissue (15) suggests an inadequacy in the oxidative mechanism. A deficiency of one of the components of the oxidative mechanism, cytochrome *c*, was demonstrated in the first paper of this series (6).

The present article reports the decreased activities of succinic dehydrogenase and of cytochrome oxidase in tumor tissues. These enzymes together with cytochrome *c* constitute the known biocatalysts of the succinoxidase system. In this system succinic acid is oxidized to fumaric acid by the catalytic removal of two atoms of hydrogen from each mole of succinate. The electrons from this hydrogen are transferred to oxidized cytochrome *c* *via* succinic dehydrogenase and from the reduced cytochrome *c* to molecular oxygen *via* cytochrome oxidase.

As was emphasized previously (6) a deficiency of cytochrome *c* alone could account for the accumulation of lactic acid in tumor tissue. The present demonstration of a deficiency of succinic dehydrogenase and of cytochrome oxidase in tumor tissue provides further basis for lactate accumulation since the succinoxidase system is an integral part of the normal oxidative mechanism (the Krebs citric acid cycle). A decrease in the activity of this system would necessarily result in a decreased activity of the whole cycle since the reaction rate of the cycle is determined by the reaction rate of the slowest system in the cycle. The question naturally arises whether the components of this system are sufficiently decreased to account for the Q_{O_2} of tumor tissue. This point will be discussed later in more detail.

Numerous papers have appeared describing succinic dehydrogenase and cytochrome oxidase activities of various tissues. The most extensive of these reports was that of Elliott and Greig (8). More recently Craig, Bassett, and Salter (3) reported succinoxidase and cytochrome oxidase determinations on slices of homologous normal and tumor tissues. Both groups demonstrated diminished activities of these enzymes in tumor tissues. Breusch (2) found that tumors were low in succinic dehydrogenase activity, whereas Stotz (13), on the other hand, observed little difference between the cytochrome oxidase activities of rat tumor R-256 and of a spontaneous rat tumor and the cytochrome oxidase activity of the normal tissues that he studied.

MATERIALS AND METHODS

Tissues were assayed for succinoxidase and cytochrome oxidase activity by methods that will be published in detail elsewhere. The succinoxidase assay is a valid measure of succinic dehydrogenase activity because the latter is the rate-determining component of the succinoxidase system in the presence of an excess of cytochrome *c* and of cytochrome oxidase. An excess of cytochrome *c* is assured by fortifying the system with the pure compound (10). An excess of cytochrome oxidase was found to exist in all the tissues studied (see the Q_{ox} values in Table I).

Oxygen uptakes were measured with the conventional Warburg apparatus. Alkali was used in the center cups to absorb any carbon dioxide that might be evolved during the reactions. The main compartment of the flasks contained 3.0 ml. of fluid; 1.0 ml. of this fluid was 0.1 *M* NaH_2PO_4-NaOH buffer at pH 7.4. The remaining components of the flasks were as follows:—

Succinoxidase system.—Three-tenths milliliter 0.5 *M* succinate pH 7.4, 0.1 ml. 4×10^{-4} *M* cytochrome *c*, 0.3 ml. 4×10^{-3} *M* $CaCl_2$, 0.3 ml. 4×10^{-3} *M* $AlCl_3$, plus homogenized tissue and enough water to make 3.0 ml.

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TABLE I. THE SUCCINIC DEHYDROGENASE (Q_S) AND CYTOCHROME OXIDASE (Q_{OX}) CONTENT OF NORMAL AND CANCER TISSUES

Q_S and Q_{OX} are obtained by measuring the Q_{O_2} (cu. mm. O_2 per mgm. dry weight per hour) with succinate and ascorbate as the respective substrates, and in the presence of an excess of cytochrome *c*. See text.

Tissue	Etiology	Number of samples	Q_S Average	Q_S Range	Q_S From (8)	Q_{OX} Average	Q_{OX} Range	Q_{OX} Corrected *	Q_{OX} From (8)	$\frac{Q_{OX}}{Q_S}$
Rat heart	Normal	6	219	197 250	62	974	693 1170	1699	506	4.4
Rat kidney	Normal	6	195	174 226	112	549	511 585	695	288	2.8
Rat liver	Normal	7	87.7	76.8 101	66	392	281 518	479	167	4.5
Rat brain	Normal	6	48.7	41.0 64.1	18	420	306 584	476	134	8.6
Rat muscle	Normal	6	35.5	29.2 48.7	6.6	180	110 274	271	38	5.1
Rat spleen	Normal	6	23.3	19.4 35.3	0.5	195	158 317	222	32	8.4
Rat lung	Normal	6	17.9	15.0 21.6	7.5	92.3	70.6 114	109	31	5.2
Rat liver tumor	Orally ingested BY §	4	26.3	19.4 40.8	—	134	99.5 187	151	—	5.1
Rat liver tumor †	Orally ingested BY §	6	25.0	20.6 32.8	—	67.2	54.0 87.1	—	—	2.7
Hepatoma 31 ‡ rat	Originally oral BY §	7	21.7	17.1 26.0	—	136	120 153	163	—	6.3
Transplantable hepatoma	Originally oral BY §	4	18.1	15.7 20.3	—	124	103 141	—	—	6.9
Walker 256 rat carcinosarcoma	Originally spontaneous	6	9.4	7.7 12.0	—	61.5	49.2 74.6	80.2	—	6.6
Walker 256 † rat carcinosarcoma	Originally spontaneous	10	12.3	7.1 14.9	0.6 7.2	77.8	60.7 105	—	15	6.3
Flexner-Jobling rat carcinoma	Originally spontaneous	7	15.5	13.3 17.9	—	91.3	59.2 124	100	—	5.9
Flexner-Jobling rat carcinoma †	Originally spontaneous	10	14.8	11.9 17.7	12.8 2.1	75.9	54.4 99.0	—	28	5.2
Yale No. 1 mouse tumor	Estrin	6	20.2	18.6 22.5	—	106	92.1 129	113	—	5.2
Yale No. 1 mouse tumor †	Estrin	10	19.0	13.0 24.5	—	87.5	69.0 108	—	—	4.8
Mouse ear tumor †	Ultraviolet irradiation	10	19.1	16.0 23.7	—	64.0	51.2 78.9	—	—	3.3
Rous chicken sarcoma †	Virus	10	11.1	6.5 15.2	—	44.4	28.7 55.1	—	—	4.0
Mouse mammary tumors †	Spontaneous	10	27.7	18.9 31.9	0.5	87.8	56.8 119	—	—	3.2
Jensen rat sarcoma	Originally spontaneous	7	17.8	15.3 21.1	13	129	106 170	148	43	7.2

* The Q_{OX} values in this column were corrected for the degree of homogenization of the tissue.
† These tissues were homogenized in *M*/30 phosphate buffer at pH 7.4. All other tissues were homogenized in glass-redistilled water.
‡ Original tumor supplied through the courtesy of Dr. Carl Voegtlin.
§ BY = *p*-Dimethylaminoazobenzene.

Cytochrome oxidase system.—Three-tenths milliliter 0.114 *M* ascorbic acid neutralized to pH 7.4 with NaOH, 0.6 ml. 4×10^{-4} *M* cytochrome *c*, 0.3 ml. 4×10^{-3} *M* $AlCl_3$, plus homogenized tissue and enough water to make 3.0 ml.

The tissues were prepared by the homogenization technic (11), the amounts used depending upon the activity of the tissue. Two or more concentrations of tissue were used in each system to eliminate the possibility of a dilution effect (11, 12). In addition, one succinoxidase determination was made in the absence of added cytochrome *c*; the value of this determination as compared to a similar determination in the presence of excess cytochrome *c* was considered to be a measure of the intact cells remaining in the homogenate. This factor was then used to correct the cytochrome oxidase Q_{O_2} values because it was found that ascorbic acid was unable to penetrate intact cells whereas succinate was able to do so. The oxygen uptakes in the cytochrome oxidase assays were corrected for ascorbate autoxidation by extrapolating the values from three different tissue concentrations to zero tissue concentration. Reduced cytochrome *c* is the actual substrate for the cytochrome oxidase system; reduced cytochrome *c* was supplied in this assay by adding an excess of cytochrome *c* and of ascorbic acid to reduce the cytochrome *c*. No enzyme is needed to catalyze the reaction between ascorbate and cytochrome *c*. The roles played by calcium and aluminum have been discussed previously (12, 14).

Essentially the same types of tumors were used as in the previous report (6). Special care was taken to avoid contamination of the tumor tissue with either normal tissue or necrotic tumor. The inhibiting effect of necrotic tissue on succinoxidase and cytochrome oxidase activity has been studied recently in this laboratory (1).

RESULTS

The results of the assays of both normal and tumor tissues are presented in Table I. Seven normal tissues and ten types of tumor tissues were assayed for succinic dehydrogenase and cytochrome oxidase activity. The activities of both enzymes fell within a rather narrow range for all tumors regardless of etiology: about 10 to 25 for the succinic dehydrogenase Q_{O_2} (Q_s), and about 80 to 160 for the cytochrome oxidase Q_{O_2} (Q_{ox}). A much greater variation occurred in normal tissues: $Q_s=18$ to 219, and $Q_{ox}=92$ to 974. Normal tissues on the whole exhibited greater activity than did malignant tissues; when homologous tissues were compared, liver showed considerably greater activities of both these enzymes than did liver tumors.

Attempts were also made to demonstrate a decrease in the activity of the succinoxidase system in the liver of tumor-bearing animals as has been done for catalase (9). These experiments were negative and are therefore not reported in detail.

The results of Elliott and Greig (8) are included in Table I for purposes of comparison. The general trend of their results is in agreement with our own, though ours are, on the whole, considerably higher; this can probably be attributed to improvements in assay conditions, including the rigid exclusion of necrotic tissue when assaying tumors (1).

Comparison of the data obtained from tissues homogenized in water with those from tissues homogenized in isotonic phosphate shows that the succinoxidase activity is not appreciably affected by increasing the cell disruption (as is done in water homogenization), whereas the cytochrome oxidase activity is substantially increased. Only the Walker 256 rat carcinosarcoma failed to show an increased cytochrome oxidase activity upon homogenization in water. The increased cytochrome oxidase activities observed when tissues were homogenized in distilled water suggested that the measurement of cytochrome oxidase activity is dependent upon how well the cells are disrupted. Since no appreciable changes were observed in the succinic dehydrogenase activities in tissues homogenized in water as compared to isotonic phosphate it was concluded that succinate was able to penetrate intact cells whereas ascorbate was not. Even water homogenization was unable to cytolyze all the cells and therefore the cytochrome oxidase values that were corrected for the degree of homogenization of the tissue are probably a more accurate measure of the actual cytochrome oxidase activity in the intact cell than are the uncorrected values. However, both values are given.

DISCUSSION

The assay results seem to indicate that tumor tissues are deficient in succinic dehydrogenase and cytochrome oxidase. Some of the normal tissues, however, also have activities that are about as low as those of tumor tissues. Lung, spleen, and skeletal muscle fall within this classification. Lung and spleen do not require large amounts of oxidative enzymes since they are not called upon for rapid responses and because they have low rates of carbohydrate breakdown. Skeletal muscle, on the other hand, is a tissue in which a rapid response is required; but in this case a large excess of oxidative enzymes is unnecessary because the tissue is able to glycolyze aerobically.[1] Since tumors are considered to be active tissues a more valid method of evaluating their oxidative enzyme activity would

[1] Skeletal muscles are capable of developing high concentrations of aerobic enzymes when they are called upon for high work output; *viz.*, pigeon breast muscle.

be to compare them with normal tissues that also are very active. Heart, kidney, and liver are of this type, and comparison of them with tumor tissues shows that the latter are definitely deficient in these enzymes.

Perhaps the most valid comparison can be made between homologous tissues, such as normal liver and liver tumor. A definite deficiency of these enzymes is evident from such a comparison: Normal liver has a Q_s of 90 while liver tumor has a Q_s of only 25; normal liver has a Q_{ox} of about 480 while that of liver tumor is about 160. These results are in harmony with those reported previously for cytochrome *c* (6). The question now arises whether the deficiencies of these three enzymes are significant. It has often been demonstrated that the Q_{O_2} values, as measured *in vitro,* for normal and tumor tissues are much the same. This has been confirmed recently by the work of Craig, Bassett, and Salter (3), who reported that the Q_{O_2} of normal mouse liver on glucose was 10.5 while that of liver tumor was 10.6. Although the Q_{O_2} value as measured for the liver tumor *in vitro* may be a fair estimate of the oxidative activity of the liver tumor *in vivo,* that of the normal liver is likely to represent a resting value. The work of Deutsch and Raper (4, 5) on slices of the submaxillary gland of the cat indicates that the Q_{O_2} as measured on glucose represents only a resting value, for when the slices were stimulated *in vitro* by acetylcholine in the presence of eserine the Q_{O_2} rose considerably and reached as much as 7 times its original value. This suggests that the Q_{O_2} of liver may also vary above that of the resting level in response to the variations in functional requirements that occur *in vivo.* The Q_{O_2} of liver tumor, on the other hand, would necessarily be quite constant since tumors are considered to have only one activity, steady continuous growth that is independent of an outside stimulus. The results of Deutsch and Raper also suggest why tissues such as heart, liver, and kidney have so much succinic dehydrogenase and cytochrome oxidase; large amounts of these oxidative enzymes are needed to meet the variations in the activity of the tissue *in vivo.*

If we assume that the Q_{O_2} measured for liver tumors *in vitro* is correct, it appears that the succinoxidase system is not the limiting factor in the oxidative metabolism of tumors, because the deficiencies in the components of the succinoxidase system appear to be insufficient to account for the low Q_{O_2} in liver tumors. Further evidence for this point of view is furnished by Elliott and Greig (7), who found that succinic acid did not accumulate in tumor slices that were oxidizing pyruvate. If the succinoxidase system were the limiting factor in the oxidative mechanism of tumors, succinic acid would necessarily accumulate. Thus it may be suggested that the weakest link in the oxidative mechanism of tumor tissues lies somewhere between pyruvic acid and succinic acid. Further work will be necessary to confirm this point of view.

SUMMARY

1. The succinic dehydrogenase and the cytochrome oxidase activities have been determined for seven normal rat tissues and for ten kinds of experimental tumors.

2. The Q_{O_2} values for these enzymes in tumor tissues were considerably lower than those for normal tissues as a whole; the Q_{O_2} values in tumors were quite constant regardless of etiology, whereas in normal tissues they varied more widely.

3. Liver tumors had only about one-fourth of the succinic dehydrogenase activity and about one-third of the cytochrome oxidase activity of normal liver.

4. Homogenization of the tissue in water increased the cytochrome oxidase activity above that observed in tissues homogenized in isotonic phosphate buffer; this procedure had no appreciable effect upon the succinoxidase activity.

5. The available evidence suggests that tumor tissues do have a deficient type of oxidative metabolism but that the succinoxidase system is not the weakest link in the oxidative cycle.

REFERENCES

1. Albaum, H. G., and Potter, V. R. Biocatalysts in Cancer Tissue. II. Inhibition of the Succinoxidase System by Tumor Extracts. Cancer Research, **3**:303-308. 1943.
2. Breusch, F. L. Die quantitativen Verhältnisse von Fumarase und Succinodehydrase in verschiedenen Geweben. Biochem. Ztschr., **295**:101-106. 1937.
3. Craig, F. N., Bassett, A. M., and Salter, W. T. Artificial Benignancy of Neoplasm. VI. Observations on the Oxidative Behavior of Tumors, Artificially Benign Tumors, and Homologous Normal Tissues. Cancer Research, **1**: 869-879. 1941.
4. Deutsch, W., and Raper, H. S. Respiration and Functional Activity. J. Physiol., **87**:275-286. 1936.
5. Deutsch, W., and Raper, H. S. The Respiration and Metabolism of Submaxillary Gland Tissue of the Cat. J. Physiol., **92**:439-458. 1938.
6. DuBois, K. P., and Potter, V. R. Biocatalysts in Cancer Tissue. I. Cytochrome *c*. Cancer Research, **2**:290-293. 1942.
7. Elliott, K. A. C., and Greig, M. E. The Metabolism of Lactic and Pyruvic Acids in Normal and Tumor Tissues. IV. The Formation of Succinate. Biochem. J., **31**:1021-1032. 1937.
8. Elliott, K. A. C., and Greig, M. E. The Distribution of the Succinic Oxidase System in Animal Tissues. Biochem. J., **32**:1407-1423. 1938.
9. Greenstein, J. P., Jenrette, W. V., and White, J. The Relative Activity of Xanthine Dehydrogenase, Catalase, and Amylase in Normal and Cancerous Hepatic Tissues of the Rat. J. Nat. Cancer Inst., **2**:17-22. 1941.

10. Potter, V. R. Studies on the Mechanism of Hydrogen Transport in Animal Tissues. IV. The Succinoxidase System. J. Biol. Chem., **141**:775-787. 1941.
11. Potter, V. R., and Elvehjem, C. A. A Modified Method for the Study of Tissue Oxidations. J. Biol. Chem., **114**:495-504. 1936.
12. Potter, V. R., and Schneider, W. C. Studies on the Mechanism of Hydrogen Transport in Animal Tissues. V. Dilution Effects in the Succinoxidase System. J. Biol. Chem., **142**:543-555. 1942.
13. Stotz, E. The Estimation and Distribution of Cytochrome Oxidase and Cytochrome C in Rat Tissues. J. Biol. Chem., **131**:555-565. 1939.
14. Swingle, K. F., Axelrod, A. E., and Elvehjem, C. A. The Mechanism of the Effect of the Calcium Salts on the Succinoxidase System. J. Biol. Chem., **145**:581-591. 1942.
15. Warburg, O. Ueber den Stoffwechsel der Tumoren. Berlin-Dahlem: Springer. 1926.

Biocatalysts in Cancer Tissue

IV. An Enzyme-Virus Theory Regarding Carcinogenesis

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In experiments on the additive effects of certain carcinogens, Lavik, Moore, Rusch, and Baumann (13) showed that mice pretreated with a given hydrocarbon developed subcarcinogenic changes in the tissue. The authors suggested that in various animals no tumor cells were present as such following the pretreatment, but only precancerous or "part-tumor" cells. To explain this anomalous situation it was suggested that "the significant change in carcinogenesis is an alteration in tissue protein, possibly in nucleoprotein or chromatin." Similar studies have also been published by Rous and Kidd (20) and by MacKenzie and Rous (14), who refer to "subthreshold neoplastic states" or "latent neoplastic changes."

We have studied the cancer problem in terms of the enzymatic components of normal and cancer tissue (8, 16, 18, 19, 21), and it is our purpose to describe at this point a theory of cancer formation that we believe will prove helpful in explaining the meaning of such terms as "latent neoplastic changes" in terms of changes in the enzymatic make-up of the tissues involved. The theory is in complete agreement with the "altered protein" concept (13), but it emphasizes the idea that the protein that becomes altered is an *enzyme* or a complex of enzymes. This modification of the idea is extremely important from the standpoint of methodology. It is felt that the identification of the altered protein will prove to be a problem in enzyme chemistry.

According to the theory, the protein that becomes altered is considered to be an enzyme, which, pending identification, is referred to as enzyme X, and the *altered protein* is referred to as "cancer virus." [1] The relations between the two principal foci of the theory are shown in Fig. 1, and the theory may be stated as follows:—

1. Cancer is considered to result from the introduction of an abnormal protein (called "cancer virus" in Fig. 1) into organized tissue. This protein could arise in a variety of ways and could take a number of forms, but in every case certain characteristic features would be found, as indicated below.

2. The "cancer virus" is assumed to be almost identical with enzyme X, except that it lacks the specific catalytic potency of this enzyme. The identity of enzyme X could vary in different types of cancer.

3. Both the "cancer virus" and enzyme X are assumed to require essentially the same building blocks for their synthesis, and the characteristics of carcinogenesis may be explained in large part on the basis of competition between the "cancer virus" and enzyme X for the building blocks, which might include amino acids, vitamins, and minerals for prosthetic groups, as well as phosphate. The latter would need to be esterified to energize endergonic syntheses (11) but might need to be free to serve as a building block. The cancer-inhibiting effect of various dietary restrictions may well be related to the competition between enzyme X and the "cancer virus" for building blocks.

4. The synthesis of both enzyme X and the "cancer virus" is assumed to proceed autosynthetically.[2] Thus, the results of the competition between the two proteins are assumed to depend upon their relative concentrations.[3] The "sudden" appearance of cancer would be explainable on this basis.

[1] The term "cancer virus" is enclosed in quotes because, by definition, the altered protein cannot be called a virus until it has been isolated and shown to transmit the characteristic malignant change. The recent production of mouse tumors by cell-free extracts (22) gives renewed emphasis to the possibility that cancers in general may be produced by viruses.

[2] In a growing cell in which enzyme X is being synthesized, one may as well assume that the enzyme grows by autosynthesis as to assume that it is synthesized by an enzyme X_n which, in turn, is formed autosynthetically.

[3] Points 1 to 4 may be applicable to viruses in general. We regard a virus as a protein, the constitution of which resembles an enzyme X so closely that it competes successfully for the constituent parts common to them both. The synthesis of both undoubtedly requires coupled energy transfers (11) that derive their energy from the glycolytic or respiratory systems of the cell. The virus may be regarded as a pseudoenzyme or a misplaced enzyme, and the simplest viruses are probably analogous to a simple enzyme system. More complex viruses probably consist of several associated "enzymes." The transition from "lifeless" to "living" would occur when the group of associated "enzymes," the synthesis of which as individuals depended upon the coupled energy transformations of a host enzyme system, become self-contained and organized to obtain energy for themselves.

5. From the above considerations, it would follow that a "cancer cell" would result whenever the ratio of "cancer virus" to enzyme X attained a value such that the synthesis of enzyme X could no longer compete successfully for the building blocks.

6. It is assumed that both enzyme X and "cancer virus" are broken down in time, with antigen-antibody reactions playing an important role in the case of the latter. If the disposal of the "cancer virus" takes place rapidly enough, the competition with enzyme X is unsuccessful and no cancer cells result.

virus is assumed to be the same as that of the altered proteins produced by carcinogens, that is, a competition with enzyme X, which, when successful, results in a "cancer cell."

9. Growth is assumed to be correlated with a lowering in the effective concentration of enzyme X. This correlation might conceivably be mediated by changes in the concentration of inorganic phosphate or other breakdown products of adenosine triphosphate.

In order to explain the possibilities of such a mechanism, it is necessary to introduce the modern concepts

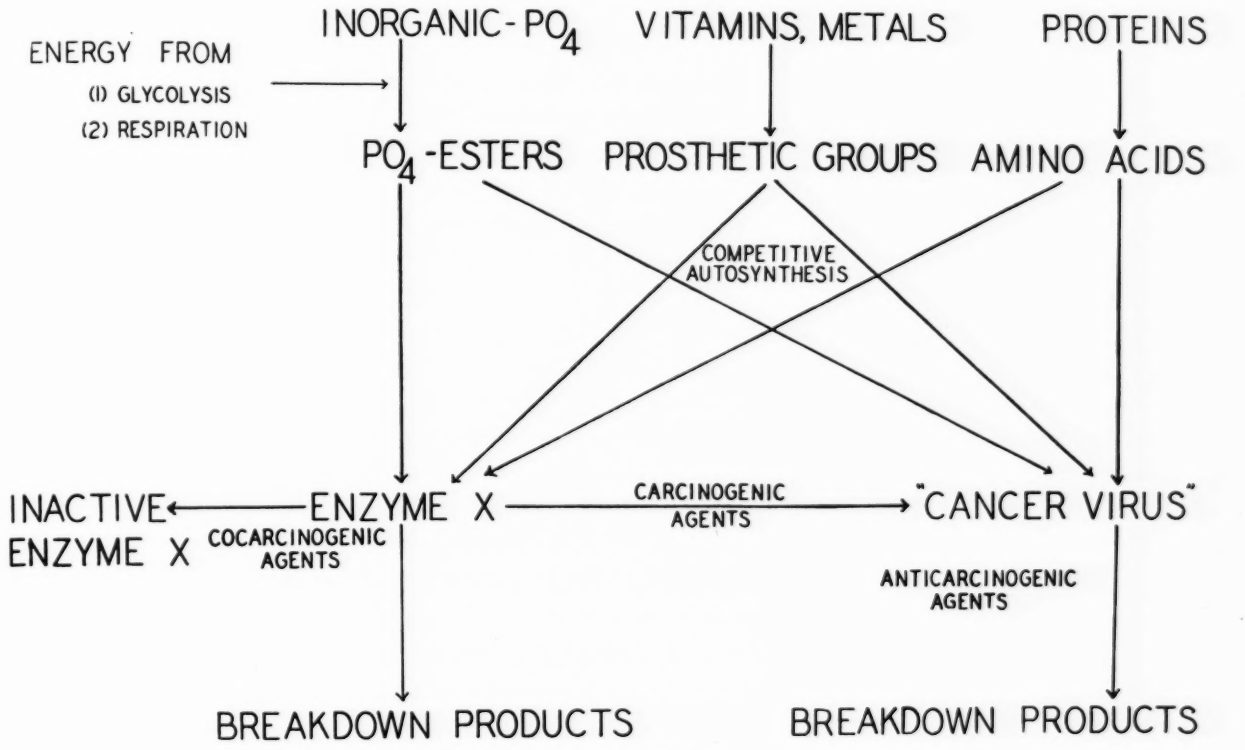

FIG. 1.—Working hypothesis for studying the cancer problem in terms of enzymes.

7. According to the above concepts, a *carcinogen* would be any agent that would convert enzyme X into "cancer virus," and a *cocarcinogen* would be an agent that could convert enzyme X into an inactive form but not into the "cancer virus" and that, in addition, would not damage the latter. The cocarcinogen would thus act by changing the ratio in favor of the "cancer virus." Cocarcinogens might also act by increasing the concentration of inorganic phosphate through the breakdown of adenosine triphosphate, as indicated in paragraph 9.

8. In certain cases the "cancer virus" can be isolated and recognized as a true virus (*e.g.*, Rous virus), but the mechanism of action in the case of the isolated

of transphosphorylation and phosphate esterification, which have been excellently described by Kalckar (11). It is now known that phosphate plays a key role in the management of the cellular economy. Johnson (9) has shown that the acceleration, deceleration, and reversal of glycolysis can be explained in terms of the release of inorganic phosphate associated with metabolic work. According to this explanation, glycolysis proceeds very slowly in resting muscle because the concentration of inorganic phosphate is so low that the stages in glycolysis involving phosphate esterification (reactions 1 and 2) are impeded by the operation of the law of mass action, thus impeding the over-all rate of glycolysis:

$$\text{glycogen} + \text{inorganic phosphate} \longrightarrow \text{glucose-1-phosphate} \quad [1]$$

$$\text{glyceraldehyde-3-phosphate} + \text{inorganic phosphate} \longrightarrow \text{glyceraldehyde-1,3-diphosphate} \quad [2]$$

When the muscle is stimulated, adenosine triphosphate is broken down to furnish energy for the contraction, and inorganic phosphate is released in amounts proportional to the work done. Glycolysis is then also accelerated proportionately, since the liberated phosphate drives reactions 1 and 2 to the right, yielding the phosphate esters. Subsequent stages in glycolysis and respiration transfer the esterified phosphate to adenylic acid, resynthesizing adenosine triphosphate for further energy-requiring reactions.[4] The uptake of inorganic phosphate represented by reactions 1 and 2 leads to the net esterification of 2 moles of phosphate per mole of glucose and 3 *n* moles of phosphate per mole of glycogen, where *n* is the moles of glucose per mole of glycogen. This is the net energy available from glycolysis. It has now been shown by a number of workers (3, 6, 10) that the *oxidation* of the end products of glycolysis results in the esterification of at least 10 moles of phosphate per mole of glucose. Furthermore, there is evidence to indicate that the aerobic phosphorylations can proceed at phosphate concentrations so low as to stop or even to reverse glycolysis at the points represented by reactions 1 and 2.[5] Indeed, this is implicit in the elegant demonstration of the reversal of reaction 1 coupled with the conversion of glucose to glucose-6-phosphate, recently published by Colowick and Sutherland (7), and it constitutes the Johnson explanation of the mechanism of the Pasteur effect (inhibition of glycolysis by oxygen).

There is thus good reason to hypothesize that the high glycolysis of tumor tissue is associated with an unrestrained adenosine triphosphate breakdown that yields energy for growth and, at the same time, provides sufficient phosphate to drive reactions 1 and 2 at appreciable rates. Evidently the aerobic mechanisms are unable to keep pace with the adenosine triphosphate breakdown, and are thus unable to esterify the inorganic phosphate rapidly enough to have any appreciable slowing action on reactions 1 and 2. Now, if the concentration of aerobic enzymes were increased sufficiently, the result would be increased removal of free inorganic phosphate and finally a slowing of glycolysis. It is apparent that growth requires not only energy for synthesis but also large amounts of phosphate for the synthesis of both nuclear and cytoplasmic nucleoproteins. Furthermore, since the enzymes making up the aerobic mechanism contain phosphate (17, Fig. 2) it is apparent that, if the synthesis of these enzymes is conditioned by the amount of available phosphate, it is possible to conceive of the regulation of growth by an autocompensatory process analogous to the mechanism for the regulation of glycolysis. That is, a lowering in the concentration of the aerobic mechanism will result in the accumulation of inorganic phosphate that will make possible the synthesis of more of the aerobic mechanism; this, in turn, will reduce the concentration of inorganic phosphate and thus prevent further synthesis. The exact operation of this mechanism is not essential to the main theory, but a possible mechanism has been introduced to show what is meant by the correlation between growth and a lowering in enzyme X. According to this explanation, enzyme X would be an aerobic enzyme.

In embryonic or healing tissue the concentration of enzyme X should be low, but is assumed to be slowly building up during the growth process. As soon as the concentration of enzyme X attained its proper level, its further increase could be stopped by the same mechanism that stopped further growth. A possible mechanism for this effect has been outlined above in terms of phosphate concentration. By contrast, in malignant tissue the concentration of enzyme X could never build up, owing to the competition by the "cancer virus," and the same process that is set off in normal growing tissue by the deficiency in enzyme X would continue indefinitely in cancer tissue. The explanation above would account for the unchecked growth in cancer tissue as well as the cocarcinogenic effect of wounds and irritations (14, 20).

The suggested theory is in no sense complete, but is described at this time to show that it is possible to formulate a mechanism of carcinogenesis that is in harmony with available facts. As new facts are added the theory can be modified, but the chief value of any theory is its ability to energize experimentation, and the only test of its accuracy is its ability to predict the results of experiments. If this theory is approximately correct, then it seems possible that measurements of R. Q. and Q_{O_2} have less significance than was formerly thought. The primary object becomes the identification of enzyme X. Attempts in this direction may be facilitated by considering the possible properties of the enzyme. It should be deficient in cancer tissue, and it should be damaged by carcinogens and cocarcinogens or their metabolic products. It would probably not be a glycolytic enzyme but might be part of the aerobic mechanism. On the basis of Claude's brilliant studies with Rous tumor virus (4) and the particulate components of cytoplasm (5, 18), one might expect enzyme X to be a ribonucleoprotein

[4] As Johnson points out, "The yeast cell seems able to utilize energy of phosphorylation for every energy requirement of its metabolism," and we assume that the energy of phosphorylation is used for cancer growth as well as for yeast growth.

[5] The rate and direction of reaction 2 are also affected by the O-R potential and the pH of the medium, as well as by the inorganic phosphate concentration, as shown by the data of Warburg and Christian (23).

of macromolecular dimensions with several associated enzyme moieties.

When these various criteria are applied to succinic dehydrogenase, the enzyme with which we are most familiar, it is found that the enzyme is deficient in tumor tissues (21); that it is damaged by the breakdown products of the carcinogen, *p*-dimethylaminoazobenzene (16, 19); that it is associated with the particulate components of cytoplasm (15), which Claude has identified as ribonucleoproteins (5); and that it is part of the aerobic mechanism. Furthermore, it is inhibited by ribonuclease (18), and it may be a flavoprotein (1, 2). This would explain the beneficial effects of feeding flavin and protein in *p*-dimethylaminoazobenzene experiments (12). We do not here propose that enzyme X is succinic dehydrogenase. The correlations are mentioned merely to illustrate the means by which the theory is being tested, and to show that at least one enzyme that will fit the various requirements of enzyme X is already available.

SUMMARY

1. A theory of carcinogenesis is proposed, suggesting that cancer may be the result of a competition between an enzyme X and a "cancer virus," the latter being derived from the former by action of carcinogenic agents. The identification of enzyme X is considered to be of prime importance in the solution of the cancer problem.

2. Some of the hypothetical properties of enzyme X are compared with the observed properties of succinic dehydrogenase.

REFERENCES

1. Axelrod, A. E., Potter, V. R., and Elvehjem, C. A. The Succinoxidase System in Riboflavin-Deficient Rats. J. Biol. Chem., **142**:85-87. 1942.
2. Axelrod, A. E., Swingle, K. F., and Elvehjem, C. A. Studies on the Succinoxidase System of Rat Liver in Riboflavin Deficiency. J. Biol. Chem., **145**:297-307. 1942.
3. Belitser, V. A., and Tsybakova, E. T. The Mechanism of Phosphorylation as Related to Respiration. Biokhimiya, **4**:516-535. 1939.
4. Claude, A. Chemical Composition of the Tumor-Producing Fraction of Chicken Tumor I. Science, **90**:213-214. 1939.
5. Claude, A. Particulate Components of Normal and Tumor Cells. Science, **91**:77-78. 1940.
6. Colowick, S. P., Kalckar, H. M., and Cori, C. F. Glucose Phosphorylation and Oxidation in Cell-Free Extracts. J. Biol. Chem., **137**:343-356. 1942.
7. Colowick, S. P., and Sutherland, E. W. Polysaccharide Synthesis from Glucose by Means of Purified Enzymes. J. Biol. Chem., **144**:423-437. 1942.
8. DuBois, K. P., and Potter, V. R. Biocatalysts in Cancer Tissue. I. Cytochrome *c*. Cancer Research, **2**:290-293. 1942.
9. Johnson, M. J. The Role of Aerobic Phosphorylation in the Pasteur Effect. Science, **94**:200-202. 1941.
10. Kalckar, H. Coupling between Phosphorylations and Oxidations in Kidney Extracts. Enzymologia, **6**:209-212. 1939.
11. Kalckar, H. M. The Nature of the Energetic Coupling in Biological Syntheses. Chem. Rev., **28**:71-178. 1941.
12. Kensler, C. J., Suguira, K., Young, N. F., Halter, C. R., and Rhoads, C. P. Partial Protection of Rats by Riboflavin with Casein against Liver Cancer Caused by Dimethylaminoazobenzene. Science, **93**:308-310. 1941.
13. Lavik, P. S., Moore, P. R., Rusch, H. P., and Baumann, C. A. Some Additive Effects of Carcinogenic Hydrocarbons. Cancer Research, **2**:189-192. 1942.
14. MacKenzie, I., and Rous, P. The Experimental Disclosure of Latent Neoplastic Changes in Tarred Skin. J. Exper. Med., **73**:391-416. 1941.
15. Potter, V. R. Studies on the Mechanism of Hydrogen Transport in Animal Tissues. IV. The Succinoxidase System. J. Biol. Chem., **141**:775-787. 1941.
16. Potter, V. R. The Inhibition of Sulfhydryl-Containing Enzymes by Split Products of *p*-Dimethylaminoazobenzene. Cancer Research, **2**:688-693. 1942.
17. Potter, V. R. The Role of Vitamins in Energy Transformations. J. Am. Diet. Assn., in press.
18. Potter, V. R., and Albaum, H. G. Studies on the Mechanism of Hydrogen Transport in Animal Tissues. VII. Inhibition by Ribonuclease. J. Gen. Physiol., **26**:443-455. 1943.
19. Potter, V. R., and DuBois, K. P. Studies on the Mechanism of Hydrogen Transport in Animal Tissues. VI. Inhibitor Studies with Succinic Dehydrogenase. J. Gen. Physiol., **26**:391-404. 1943.
20. Rous, P., and Kidd, J. G. Conditional Neoplasms and Subthreshold Neoplastic States. A Study of the Tar Tumors of Rabbits. J. Exper. Med., **73**:365-390. 1941.
21. Schneider, W. C., and Potter, V. R. Biocatalysts in Cancer Tissue. III. The Succinoxidase System. Cancer Research, **3**:353-357. 1943.
22. Taylor, A. The Successful Production of a Mammalian Tumor with a Virus-Like Principle. Science, **97**:123. 1943.
23. Warburg, O., and Christian, W. Isolierung und Kristallisation des Proteins des oxydierenden Gärungsferments. Biochem. Ztschr., **303**:40-68. 1939.

Fluorescence Studies of Carcinogens in Skin*

I. Histological Localization of 20-Methylcholanthrene in Mouse Skin after a Single Application

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In the integrative study of the production of cancer in the epidermis of mice by treatment with methylcholanthrene, it is essential to relate the methylcholanthrene to the developing lesions as accurately as possible. For this reason an effort is herein described to follow the course of this carcinogen through the different parts of the skin. The strong characteristic fluorescence of methylcholanthrene suggests that this problem can be approached with the technic of fluorescence microscopy. Recent investigations in this laboratory have shown that in Swiss strain mice a single cutaneous application of approximately 0.3 mgm. of 20-methylcholanthrene in benzene solution is sufficient to induce a pronounced hyperplasia of the epidermis that leads to the development of carcinomas in a considerable fraction of the treated animals (4, 5). Definite chemical changes are demonstrable in the skin within a few days following this treatment (2, 9). These findings require a revision of the commonly accepted idea that chemical carcinogenesis is due to the repeated and prolonged stimulation of epidermal cells by externally applied chemical carcinogens, and make the study of changes following a single application of a carcinogen of great interest.

Using this method we have studied the absorption, distribution, and persistence of methylcholanthrene in the skin of mice following a single application of the carcinogen in benzene solution. So far as we are aware no previous work has been reported on the exact localization of carcinogens applied to the skin, though Beck and Peacock (1) and Hieger (8) report using ultraviolet light to determine by gross examination the persistence of fluorescence on the surface of mouse skin.

MATERIALS AND METHODS

Observations of microscopic fluorescence reported here were made on approximately forty 3 to 4 month old mice of the Swiss strain. Routine macroscopic examinations for fluorescence of painted skin have been made on well over a hundred animals.

A 0.6 per cent solution of methylcholanthrene in reagent grade benzene was applied by means of a No. 4 camel's hair brush to an unepilated skin area stretching from the interscapular region to the middle of the back. In some cases 1 brush stroke was applied; in most 3 were used. Each stroke delivered approximately 0.1 mgm. of the carcinogen. For microscopic preparations mice were killed at intervals up to 2 weeks after the applications. Some macroscopic observations were made also on mice that had been shaved in the area to be painted.

For fluorescence studies, tissues were fixed for 12 to 24 hours in a 10 per cent solution of neutral formalin, washed in distilled water, and cut immediately on the freezing microtome at 10 to 20 microns thickness. These sections were floated onto slides of corex D ultraviolet transmitting glass, then drained, cleared in anhydrous glycerol, and studied with the fluorescence microscope. The solubility of the carcinogen made it necessary to avoid all lipid solvents in preparation of these sections. It is relatively insoluble, however, in glycerol. Other frozen sections were stained with hematoxylin or methylene blue for orientation and with sudan IV for the study of lipid distribution. For routine histological examination tissues were fixed in 10 per cent formalin, Bouin's fluid, or Zenker-formol solution. Detailed studies of lipid distribution were made on tissues prepared by the Schridde method or by fixation in 10 per cent formalin followed by treatment for 2 days in 2.5 per cent potassium bichromate and for 2 days in 2 per cent osmic acid. We shall refer to this latter method as a modified Schridde technic. These preparations were either mounted unstained or bleached slightly and stained with Heidenhain's iron alum hematoxylin.

A General Electric Company type BH4 mercury vapor lamp in an aluminum reflector was operated on the proper autotransformer to provide a source of filtered ultraviolet light for macroscopic observations of fluorescence. This lamp has a red-purple glass bulb that absorbs most of the visible light and passes the near ultraviolet. The peak transmitted radiation is at

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about 3,650 Å. Observations were made in a darkened room.

As a light source for the ultraviolet fluorescence microscope a General Electric Company type AH4 mercury vapor lamp was employed, operated on the same auxiliary equipment as the type BH4. To remove the visible portion of the spectrum the light was filtered through a cell of either corex D or pyrex glass, containing a saturated aqueous solution of copper sulfate, and through a No. 5840 Corning glass filter. A Zeiss quartz condenser was fitted onto a standard microscope with a monocular body tube for the fluorescence studies. Most satisfactory results were obtained by using the microscope horizontally on a high table and eliminating all reflecting surfaces between the light and the microscope. An image of the capillary light source was focussed in the condenser with a quartz lens and the condenser was then adjusted to image the aperture of the quartz lens onto the preparation. This arrangement provided uniform illumination of even low power fields. A Wratten 2A gelatin filter was cut and fitted into the eyepiece of the microscope to absorb the near ultraviolet and visible deep violet that is passed by the microscope objectives.

The same type of equipment as described for microscopic observations was used in conjunction with a Leica camera (with lens removed), a 120 mm. extension tube, and a Sliding-Focussing attachment to photograph fluorescence phenomena in sections. With a 16 mm. apochromatic objective and 4× compensating ocular, images of the fluorescence were brilliant enough in most cases to permit direct focussing on the ground glass of the Leica Sliding-Focussing attachment. When this was not possible, the image was focussed with visible light and then the ultraviolet filter was put into place for photography. With Eastman Kodak Company 35 mm. panatomic X film and this apparatus exposures for methylcholanthrene fluorescence in tissue were usually of the order of only 10 to 30 seconds, although photographs of the much weaker fluorescence of untreated skin required much longer exposures. With this fine grained film no loss of resolution was apparent when 5×7 inch enlargements were made from the original Leica frames. The enlarged pictures thus produced represent an ultimate magnification of approximately 130 times.

Macroscopic Observations

The fluorescence of methylcholanthrene in ultraviolet light is too well known to require a lengthy description. The dry carcinogen emits a brilliant yellow-green fluorescence; solutions in benzene, acetone, lard, and anhydrous lanolin show a strong blue-violet fluorescence in filtered ultraviolet light. The intensity of the fluorescence in general decreases with a decrease of concentration, though at a dilution of one part of methylcholanthrene to a billion parts of benzene blue-violet fluorescence is still easily detectable.

The extent of spread of a benzene solution of methylcholanthrene on mouse skin can be determined readily by making the application in a darkened room in the light of the BH4 mercury vapor lamp. Within a few seconds of the painting the blue-violet fluorescence of the applied benzene solution changes to yellow-green, indicating that the solvent has evaporated and that the dry carcinogen is present on the hair of the animal. The time of persistence of this yellow-green fluorescence is variable and depends to some extent on the amount of carcinogen applied. Three brush strokes, delivering 0.3 mgm. of methylcholanthrene in benzene, usually produce epilation of the painted area of skin of Swiss mice in from 5 to 8 days. As epilation progresses it can be seen that most of the yellow-green fluorescence leaves the surface of the skin and in its place a strongly blue-violet fluorescent crust appears. When animals are examined in ultraviolet light 4 to 6 days after painting, one can often observe that a flake of this crust comes away from the skin with a clump of hair as it falls out. When epilation is complete the skin surface ordinarily shows only a few scattered plaques of blue-violet fluorescence. These usually scale off by 10 days to 2 weeks after the application.

Observations on shaved mice confirm the finding that the yellow-green fluorescence disappears and is replaced by a strong blue-violet fluorescence. In such animals, where observation is not hampered by the presence of hair, this change may begin as early as 2 days after the painting and may continue up to a week or, rarely, to 10 days.

Microscopic Observations

Fluorescence of the normal mouse skin.—The fluorescence of the normal mouse skin, while weak, is sufficiently evident so that one can, when the eyes are dark-adapted, readily identify the major structures within it, and even photograph the fluorescence (Fig. 2). The lower border of the epithelium is dark compared with the bright fluorescence of the dermis, so that the boundary stands out clearly. The upper portion of the epithelium fluoresces a deep blue, and even a grayish blue on the surface if an appreciable layer of keratin is present. The same deep blue is seen in the epithelium of the hair follicles. Within the dermis the most brilliant fluorescence is due to elastic fibers, which appear as twisted, bright yellowish gray lines. Collagenous fibers are more difficult to see individually, their fluorescence being a dim bluish gray. In longitudinal sections of skin from the back of the mouse the arrector pili muscles are distinguished by their

bluish green fluorescence. In these, as in the epithelium, the nuclei are nonfluorescent and appear as optically empty spaces. The fluorescence of the fat in the dermis and beneath it is variable. We have not observed in the mouse the fading green fluorescence of vitamin A described by Cornbleet and Popper (3). Some fat appears to have no fluorescence and other droplets vary from deep blue to green and even greenish yellow. These continue to fluoresce after prolonged exposure to ultraviolet light, in contrast to the vitamin A droplets of liver and intestine which fade rapidly with ultraviolet irradiation. Within the dermis

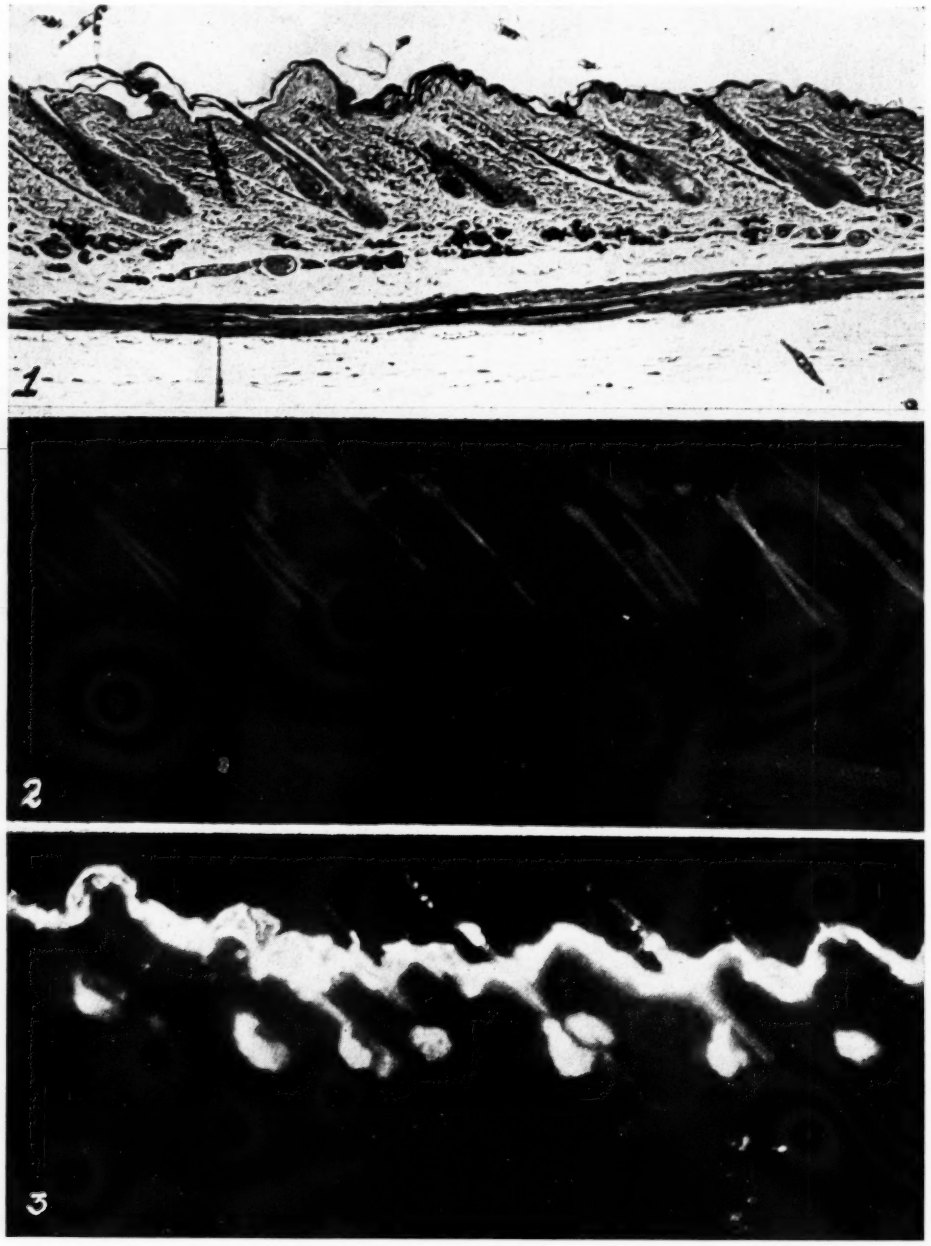


FIG. 1.—Normal skin from the back of a Swiss mouse in axial section, prepared by the modified Schridde method and counterstained with iron alum hematoxylin. Lipids of the keratinized epithelium, sebaceous glands, and subcutaneous fat cells appear black. Mag. × 130. For comparison with Figs. 2 and 3.

FIG. 2.—Similar normal skin showing the fluorescence in ultraviolet light. The tissue was fixed in 10 per cent formalin and cut at 20 microns on the freezing microtome. Because of the sensitivity of the photographic emulsion the dark blue fluorescence of the surface epithelium and sebaceous glands appears relatively stronger than it does on direct visual examination. Exposure 2 minutes. Mag. × 130.

FIG. 3.—Fluorescence of skin of animal killed 3 minutes after painting with methylcholanthrene solution. Section prepared as for Fig. 2. See text for description of colors of fluorescence. Yellow-green and blue-violet fluorescence of methylcholanthrene are not differentiated in the photograph. Intensity of fluorescence was so great that exposure time was reduced to 15 seconds for this photograph. Mag. × 130.

myelinated nerves stand out sharply by their dark blue fluorescence in ultraviolet light. Blood vessels, on the other hand, appear as empty spaces devoid of fluorescence. Within the lumen of an occasional vessel a bright fluorescent speck can be observed. At high magnifications these appear to be granular white blood cells.

Observations on skin immediately after treatment with methylcholanthrene.—Frozen sections of skin from animals killed from 3 to 5 minutes after a single application of the carcinogen showed newly acquired fluorescences that are far stronger than any occurring in normal mouse skin. Fig. 2, of normal skin, and Fig. 3, of skin 2 minutes after the painting, show this visually, although the actual difference can be better appreciated by comparing also the exposures required for photographing these two sections. The exposure for Fig. 2 was 2 minutes, that for Fig. 3 only 15 seconds.

The most striking of these changes is a brilliant blue-violet fluorescence of the sebaceous glands that allows these structures to be sharply differentiated from the enveloping dermis. Every gland appears to be filled with the fluorescent material. In the peripheral portion of the glands the fluorescent material is in the form of discrete droplets that are uniform in size within any one cell. In the midst of these droplets the nucleus is outlined as a dark circle, totally devoid of fluorescence. This distribution of fluorescent droplets corresponds to that of lipid droplets in sebaceous gland cells, which are seen as osmic acid-stained granules in Schridde preparations (Fig. 4). Toward the neck of the gland the droplets are larger and less regular and the nucleus is usually not apparent. Finally, in the acinus, neck, and duct of the gland the whole content is brilliantly fluorescent as an amorphous luminous mass and not as droplets. The color of this fluorescence appears to the unaided eye to be the same as that of methylcholanthrene dissolved in such natural lipids as lard or anhydrous lanolin.

A brilliant yellow-green fluorescence is present in and on the superficial keratinized epithelium. Similar fluorescent material encrusts parts of the exposed hair shafts. This represents, in microscopic section, the incrustation of dry carcinogen that is seen with the naked eye on the skin and hair when methylcholanthrene-painted animals are examined in filtered ultraviolet light. In the sections the methylcholanthrene often occurs in needle-like crystals, identical in shape with, though very much smaller in size than, those of the purified dry carcinogen. Beneath this yellow-green fluorescent crust is a thin layer of blue fluorescence, also much more brilliant than any fluorescence of the normal skin. Treatment of other sections with fat stains reveals that the blue fluorescence is located in the same zone and distributed in the same fashion as are the sudan IV or osmic acid-stainable lipids of the keratinized epithelium. These lipids are represented as an osmic acid-blackened layer on the surface of the skin in Fig. 1. In the rest of the epithelium the color and degree of fluorescence are the same as those seen in an unpainted epidermis.

A blue-violet fluorescence, usually less brilliant than that of the sebaceous glands, is encountered in some of the fat cells in and beneath the dermis. The number of fluorescing fat cells varies with the amount of methylcholanthrene applied. With small doses, only a few superficially situated fat cells show new fluorescence. After large doses, the superficial cells fluoresce

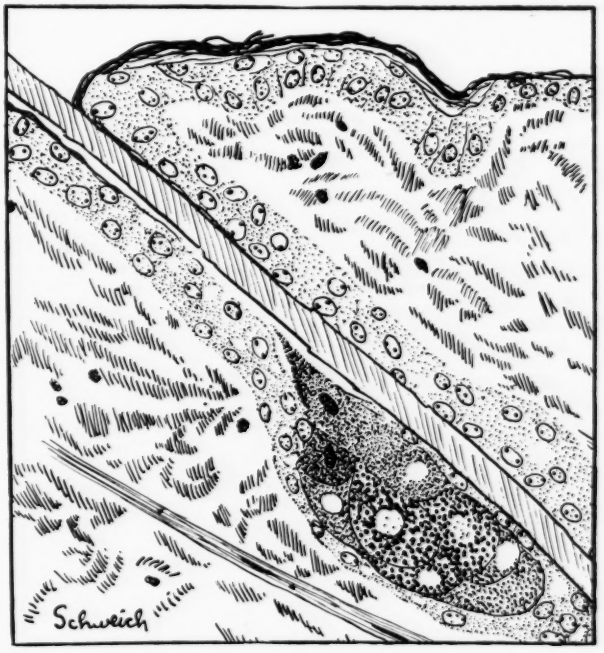


FIG. 4.—Diagrammatic representation of the normal sebaceous gland in axial skin section. The osmic acid-stained droplets correspond to the fluorescent droplets seen in the glands after treatment with methylcholanthrene.

brilliantly; those further removed from the surface exhibit a decreasing brilliance, the deepest cells showing no new fluorescence at all.

Myelinated nerves acquire in their myelin sheaths a moderately strong new blue-violet fluorescence of the same color seen in the sebaceous glands and free lipids of the keratinized epithelium. They may be traced for some distance through thick sections by this fluorescence.

The deeper layers of epidermis, dermis proper, intrafollicular hairs below the level of the sebaceous glands, epithelium of the hair follicles, arrector pili muscles, blood vessels, and lymphatics exhibit no fluorescences that do not also occur in normal mouse skin.

Persistence of the acquired fluorescences.—This is summarized in Figs. 5, 6, and 8. Animals killed up

to 24 hours after the application of methylcholanthrene show a distribution of fluorescence in the skin corresponding closely to that of animals killed immediately after the application. The only early change appears in the distribution of blue-violet fluorescence in the subcutaneous fat. The deeper fat cells acquire a part of this fluorescence at the expense of the brilliance of the more superficial fat cells, so that after about 24 hours some fluorescence is usually apparent throughout the layer of fat cells, though the superficial cells are at that time still the most brilliantly fluorescent. The skin represented in Fig. 5 has acquired little fluorescence in the fat cells, and by 24 hours this fluorescence is so diffuse that it fails to show in the photograph.

After 48 hours distinct changes begin to be apparent in other parts of the skin. The most interesting of these changes is a rapid decrease in area of the sebaceous gland fluorescence (Fig. 6). The first indication of activity in these structures is the filling of the ducts of the glands with plugs of sebum containing the fluorescent material. As these plugs are pushed up into the hair follicles it becomes obvious that the glands decrease in volume. The gland cells at the periphery still contain discrete fluorescent droplets that outline their nuclei, but the total number of cells in any gland appears to be smaller. This shrinking, once started, proceeds rapidly until, 3 to 4 days after the painting, no traces of sebaceous gland fluorescence are to be seen (Fig. 8). At the same time, the plugs of sebum, formed as the glands collapse and disappear, are pushed further out in the hair follicles. Finally, 4 to 6 days after the painting, the fluorescent sebum is completely out of the follicles and spreads over the keratinized surface of the skin. This spread of sebum over the keratin layer can be seen in the high power photograph of Fig. 10. Examination of stained sections confirms the fact that the differentiated sebaceous gland cells degenerate and disappear during this period (Figs. 7 and 9).

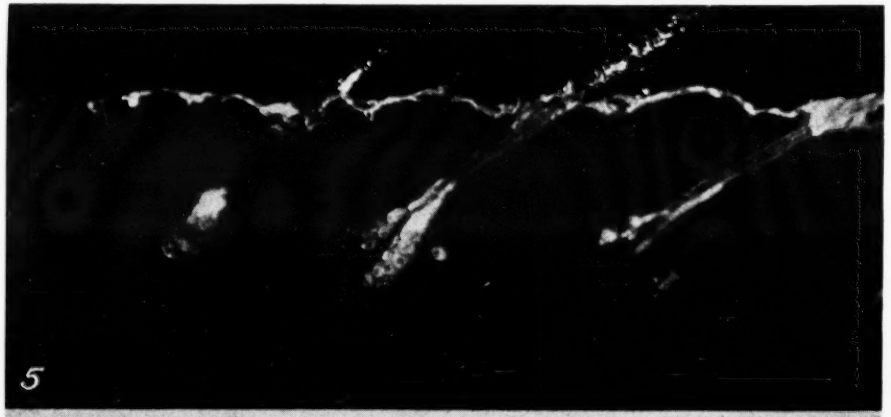
Early morphological changes in the epidermis following a single application of methylcholanthrene have been described in detail by Cramer and Stowell (6). Accompanying these are the alterations in distribution of the fluorescent substances described above. As the epithelium becomes thickened and undergoes differentiation the total amount of stainable free lipid increases in the thicker keratinized layers (compare Figs. 7 and 9 with Fig. 1). The fluorescent material is found to be more diffusely distributed in this layer, and somewhat lower in brilliance at 2 to 3 days than in earlier stages. With the outpouring of the fluorescent sebum over the surface of this thick keratin layer at 4 to 5 days, the remnants of the dry crystalline crust of methylcholanthrene go into solution. Permeation of the outer layer of horny epidermis by this solution causes the entire keratinized portion to fluoresce brilliantly blue-violet (Fig. 8). Usually this blue fluorescent crust scales off at about 5 to 8 days as the hair over the painted area falls off. Such a crust, with a hair attached, can be seen in Fig. 11. The presence of flakes like this, attached to clumps of hair, was noted macroscopically in many animals at the time epilation occurred. In these cases, which constitute a majority of the animals, no fluorescence due to methylcholanthrene could be found in the skin after the blue-violet fluorescent keratin had flaked off. The shortest time in which we observed this complete removal of the carcinogen was 4 days, the longest approximately 8 days.

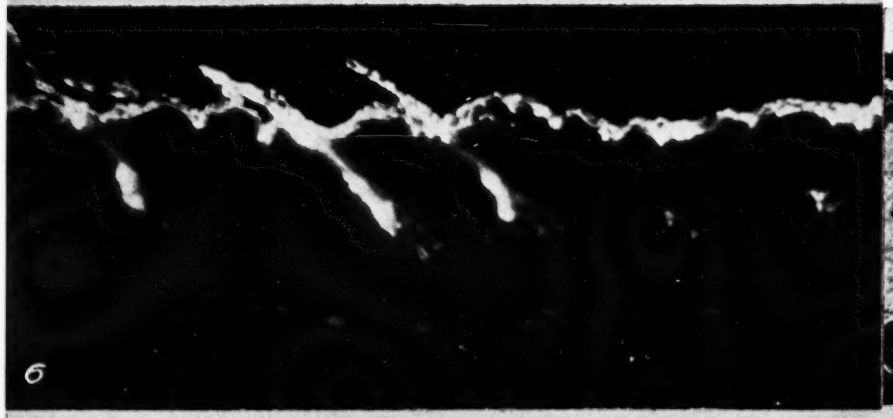
Occasionally an entirely different type of response has been seen in the epidermis. Some areas that are much thickened in response to the methylcholanthrene painting have been seen 4 to 8 days after painting. This greatly thickened, differentiated epithelium undergoes early degenerative changes, which are first indicated by the liberation of minute droplets of free lipid in the deep layers. These droplets, stainable with sudan IV and osmic acid, are found in the entire thickness of epithelium but are most prominent in the deeper layers. There is some tendency for the epithelium to separate from the dermis when sectioned. In such an epithelium brilliant blue-violet fluorescence can be seen in these lipid droplets, indicating penetration of the entire epidermis by a part of the material that is ordinarily confined to the keratinized layer. The fluorescent droplets are noted especially in the basal layer of cells in Fig. 8. Loss of this fluorescence probably occurs by further degeneration of the epidermis.

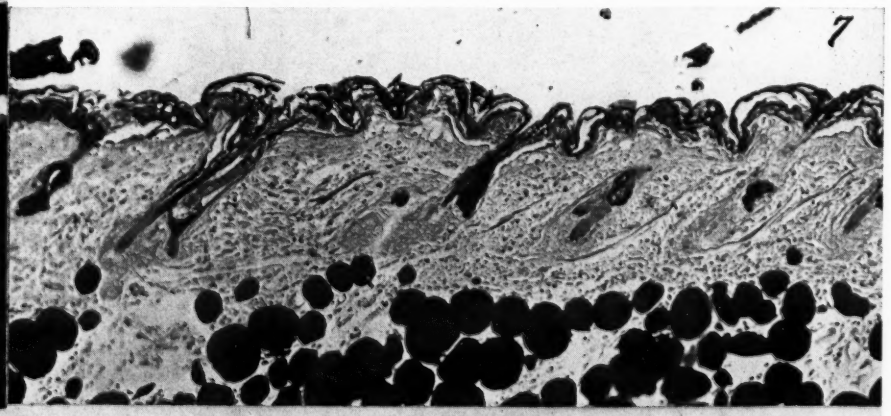
The changes in the distribution of fluorescence in the fat cells have been described up to 2 days. After that time the fluorescence tends to become more evenly distributed throughout the layer of the cells and at the same time to become gradually less brilliant. At 4 to 6 days the fluorescence is usually gone from all or nearly all these cells, the last to lose their fluorescence ordinarily being those deepest in the subcutaneous fat layer.

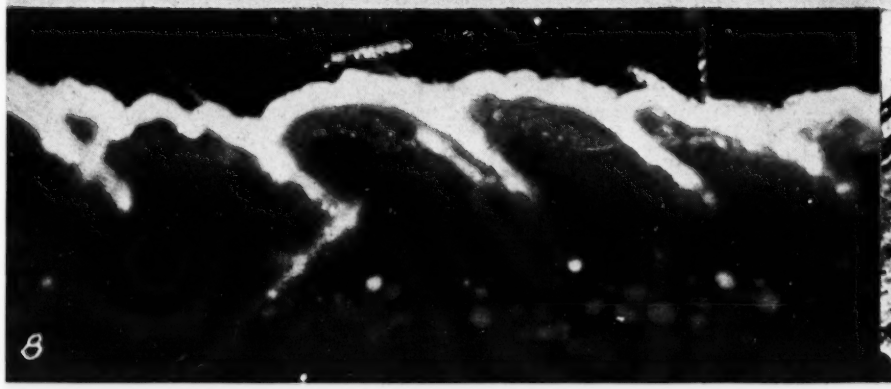
DISCUSSION

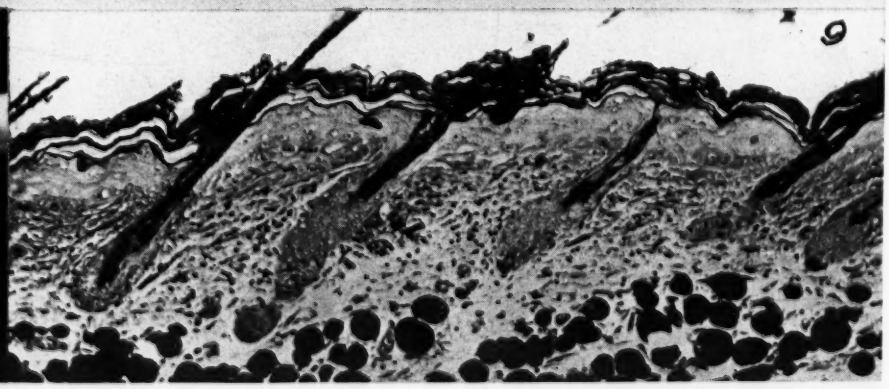
Reports on the persistence of topically applied carcinogens on skin are brief and in general agreement. They refer only to the surface of the skin and represent only naked eye observations. Beck and Peacock (1) report that fluorescence tests show 3,4-benzpyrene to disappear from the skin 4 days after one application. Hieger (8) found that, no matter how long mice were painted with 1,2,5,6-dibenzanthracene, he was unable to detect fluorescence of the compound on the skin more than 3 weeks after the last application. While these observations are of interest they throw











Figs. 5 to 9 represent successive stages after application of methylcholanthrene. Technic for Figs. 5, 6, and 8 as for Fig. 3; Figs. 7 and 9 as for Fig. 1.

Fig. 5.—One day after application. Sebaceous glands not much reduced in size. Fluorescent material distributed uniformly throughout the gland in the form of droplets. The nuclei are free from fluorescent material. Mag. × 130.

Figs. 6 and 7.—Two days after application. Glands much decreased in size. Fluorescent sebum is pushed into the hair follicles. Epidermis hyperplastic and hyperkeratinized. Mag. × 130.

Figs. 8 and 9.—Four days after application. Most glands have disappeared. Fluorescent sebum at distal end of hair follicles and in keratinized surface. Shows exceptional presence of minute fluorescent droplets in deeper layers of epidermis. Mag. × 130.

no light on where the carcinogen goes when applied nor on the persistence in those regions. Hieger has probably expressed fairly the general opinion by the

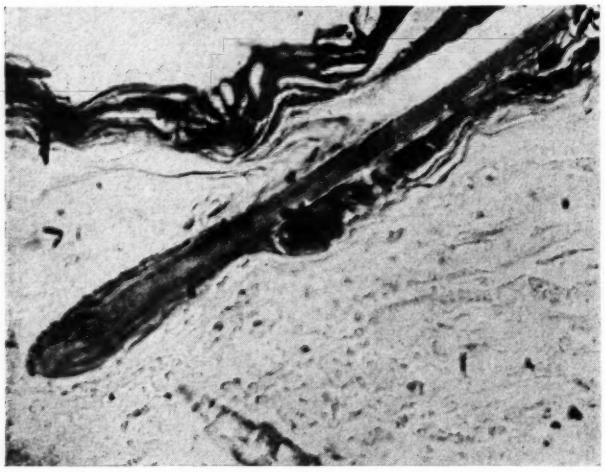


FIG. 10.—Modified Schridde preparation. Four days after application of carcinogen. Black-stained sebum at neck of hair follicle spreading over keratinized epithelium. Mag. × 530.

statement, "When applied in benzene solution to the back of a mouse from a small brush, 1:2:5:6 dibenzanthracene spreads rapidly, no doubt dissolving in the skin fat."

Our studies show, on the contrary, that the absorption of a benzene solution of methylcholanthrene is selective. One cannot rule out the possibility that very small amounts of the applied carcinogen have been absorbed in regions where no fluorescence has been observed. Carcinogen thus absorbed either must be in extremely dilute solution or so altered that its fluorescence is extinguished. There can be little doubt that all or nearly all the methylcholanthrene applied to the surface of the skin is accounted for in the sebaceous glands, lipids of the keratin layer, and the subcutaneous fat. The similarity of the blue-violet fluorescence in these structures a few minutes after painting to the fluorescence of methylcholanthrene dissolved in natural lipids, together with the fact that it is extremely unlikely that the bulk of the carcinogen could have undergone chemical change in the brief interval preceding fixation of the tissue, justifies the assumption that the fluorescence in these structures belongs to methylcholanthrene itself. Attempts are being made by spectrographic methods to validate this assumption and to study possible changes in the nature of the fluorescent substance during the time it persists in the skin.

SUMMARY

The distribution of methylcholanthrene in mouse skin during the first 10 days after a single application of the carcinogen has been studied in frozen sections by ultraviolet fluorescence microscopy. The findings have been supplemented by histological examination of frozen sections stained with sudan IV and of paraffin sections prepared by various histological methods, and by similar fluorescence and histological studies of normal mouse skin.

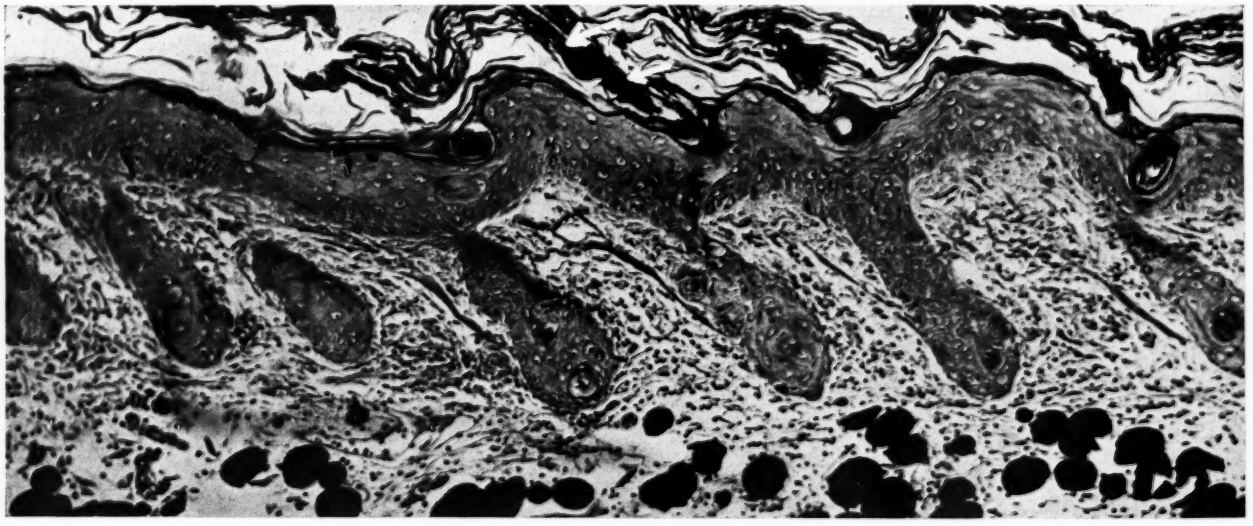


FIG. 11.—Modified Schridde and iron alum hematoxylin. Six days. Extensive hyperplasia of epidermis and hair follicles. Black-stained keratin crust with a hair attached (at arrows) can be seen separating from hyperplastic epithelium. Sebaceous glands are completely absent from such painted skin. Mag. × 130.

Immediately after the application the bulk of the carcinogen is seen in the epidermis at two special sites: in the sebaceous glands and the keratin layer. At these sites the carcinogen is dissolved respectively in the sebum and in free lipids of the keratinized epithelium. In this dissolved condition it fluoresces blue-violet. There is also on the outermost surface of the epidermis a crust of undissolved methylcholan-

threne, recognizable by its yellowish green fluorescence. The subsequent changes are described in detail. They are: (a) degeneration and disappearance of the sebaceous gland cells accompanied by a massive excretion of sebum containing methylcholanthrene from the sebaceous glands, first into the hair follicles and then onto the keratinized surface layer of the epidermis; (b) epilation; and (c) a gradual flaking off of the keratin soaked with the sebum containing the carcinogen. There is no evidence that the unchanged carcinogen is taken up directly by the epithelial cells of the normal epidermis. But in the later stages some specimens of skin show fine fluorescent globules in circumscribed areas of the epidermis where, as the result of a degenerative process, small globules of lipids are formed.

In the subcutaneous tissues the fat cells take up some methylcholanthrene and gradually lose it again. After a period of time varying from 6 to 10 days the fluorescence due to the carcinogen has disappeared from all parts of the skin.

REFERENCES

1. Beck, S., and Peacock, P. R. The Latent Carcinogenic Action of 3:4 Benzpyrene; Results of Intermittent Applications to the Skin of Mice. Brit. J. Exper. Path., **21**: 227-230. 1940.

2. Carruthers, C., and Suntzeff, V. Influence of Limited Application of Methylcholanthrene upon Epidermal Iron and Ascorbic Acid. J. Nat. Cancer Inst., **3**:217-220. 1942.

3. Cornbleet, T., and Popper, H. Properties of Human Skin Revealed by Fluorescence Microscopy. The Normal Skin; the Vitamin A Content of the Skin. Arch. Dermat. & Syph., **46**:59-65. 1942.

4. Cramer, W., and Stowell, R. E. Carcinogenesis in the Mouse's Skin by the Infrequent Application at Long Intervals of Methylcholanthrene. Cancer Research, **1**: 849-852. 1941.

5. Cramer, W., and Stowell, R. E. Skin Carcinogenesis by a Single Application of 20-Methylcholanthrene. Cancer Research, **3**:36-42. 1943.

6. Cramer, W., and Stowell, R. E. The Early Stages of Carcinogenesis by 20-Methylcholanthrene in the Skin of the Mouse. II. Microscopic Tissue Changes. J. Nat. Cancer Inst., **2**:379-402. 1942.

7. Hamperl, H. Die Fluorescenzmikroskopie menschlicher Gewebe. Virchows Arch. f. path. Anat., **292**:1-51. 1934.

8. Hieger, I. On the Mechanism of Carcinogenesis by Chemical Compounds. Am. J. Cancer, **28**:522-529. 1936.

9. Wicks, L. F., and Suntzeff, V. Reduction of Total Lipid-Protein Nitrogen Ratio of Mouse Epidermis by a Single Application of Methylcholanthrene. J. Nat. Cancer Inst., **3**:221-226. 1942.

A Spectrochemical Study of Estrogen-Induced Mammary Cancer in Mice*

I. Chemical Preparation of Tissue and Analysis by Spectrophotometry

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Little is known of the mode of action of the estrogens in carcinogenesis subsequent to their prolonged administration. The results of early studies on the metabolism and distribution of estrogens are inconsistent, probably because of variations in the methods of bioassay. Although these procedures may detect microamounts of active substances, the reaction is nonspecific; the same response may be evoked by a group of chemically unrelated substances.

More recently the methods of spectrophotometry have been applied to the assay of estrogens, the catabolism and manner of their detoxification having been deduced from the spectrographic identification of ketosteroid metabolites in extracts of urine and feces (4, 20, 21). But studies of excreta necessarily include effects produced by the metabolic action of the liver and kidneys, and there seems to have been no study of the occurrence of estrogens directly at the site of fomation of estrogen-induced tumors except by bioassay (8, 16, 19). It would be of evident value to focus the study of estrogen action at the site of carcinogenesis by direct and precise methods of analysis.

In this report are described the analytical methods we have used in studying the appearance of triphenylethylene, a synthetic estrogen, within the mammary gland itself during the process of carcinogenesis. It was necessary to devise extraction procedures that would isolate the estrogen in order to have it spectrographically measurable in the small amounts physiologically effective.

Spectrochemical Methods

The ultraviolet spectrograph as a tool.—The ultraviolet spectrograph has been increasingly recognized as a useful tool for the microanalysis of biologically significant agents. The properties of chemical unsaturation, frequently related to their physiological activity, are exactly responsible for the characteristic absorption of ultraviolet light by carcinogens and by hormones. The isolation, synthesis, and purification of many closely related carcinogenic hydrocarbons has been aided by a knowledge of their optical absorption (11, 12, 26) which, indeed, is more characteristic than most properties commonly used in identification or analysis. The spectrograph has been employed to detect and trace small amounts of administered carcinogenic hydrocarbons in tissues (15, 17, 18). Because minor changes in chemical structure, such as the addition of hydroxyl groups, are usually reflected in appreciable changes in the absorption spectrum, the metabolites and conversion products may be observed, as in the case of the carcinogenic hydrocarbon 1,2,5,6-dibenzanthracene (6, 14, 18).

In general then, the ultraviolet spectrographic method has inherent advantages as a tool for these studies: (a) Microamounts of estrogen may objectively be determined qualitatively and quantitatively. (b) The materials under investigation will probably not be destroyed in the process of analysis. (c) The results are recorded permanently on a photographic plate. (d) Changes in the spectrum represent new molecular configurations that may have resulted from metabolic changes in the organism.

Fundamental theory and technic.—The fundamental theory relating the ultraviolet optical absorption to chemical structure and the Beer-Lambert law of quantitative relationship between the measurable optical density of a solution and the concentration

$$\epsilon = \frac{1}{cd} \cdot \log \frac{I_0}{I}$$

has been adequately presented in earlier issues of this journal (5, 13, 14), and elsewhere. The limitations and pitfalls also are inferred or expressly stated. This report will refer incidentally to these details and will include, in addition, a description of a procedure

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convenient for quantitative analysis with only a Baly tube and a densitometer, as an extension of a "single beam" method previously used by one of us (23).

Apparatus.—Either a high voltage hydrogen discharge tube or a low voltage DC glow discharge tube provided a beam of ultraviolet continuous radiation. For qualitative analysis and survey purposes, a single beam was rendered parallel and passed through a test solution in a Baly tube whence it was focused on the slit of a medium flat field spectrograph (Hilger E498). The spectra were photographed on either Eastman 33 or spectrum analysis No. 1 emulsions on 4×10 inch plates. The latter has a band of depressed sensitivity centering at 3,000 Å that decreased its value for this work. A Hilger-Spekker ultraviolet photometer was used with the low voltage continuous source for accurate optical density measurements as a function of wave length. An Applied Research Laboratories microdensitometer (3) was employed in routine quantitative analyses of the estrogen in a manner to be described in a later section.

Triphenylethylene (α-phenylstilbene), m.p. 68–70° C., a synthetic estrogen, was selected because: (a) It gave the maximum absorption spectrum, *i.e.*, had the largest extinction coefficient, of the estrogens examined.[1] (b) The effective dose of triphenylethylene is much larger than that of the commonly used estrogens. These two factors are favorable for tracing this substance in tissues. Incidentally, the chemical structure of triphenylethylene is such that its metabolites should be observable spectrographically. We prepared triphenylethylene, using a Grignard synthesis, by condensation of benzyl magnesium chloride with benzophenone, dehydration, and purification by vacuum distillation and recrystallization (10). The synthesis was aided by spectrographic control. The absorption spectrum of the product synthesized by this method is in good agreement with a sample obtained from another source.[2]

The absorption spectrum of triphenylethylene in hexane was determined with a Spekker photometer and continuous light source described in the paragraph on apparatus. A comparator-densitometer was used to expedite the reading of match points with accuracy. The absorption spectrum (Fig. 1) is characterized by a symmetrical curve with maximum at 2,980 Å, $\epsilon = 2.08 \times 10^4$ ($\log \epsilon = 4.32$), and a minimum at 2,600 Å, $\epsilon = 0.74 \times 10^4$ ($\log \epsilon = 3.87$). The width of this band at half maximum extinction[3] is 500 Å. A secondary maximum develops to wave lengths shorter than 2,400 Å. The absorption curve for triphenylethylene in chloroform has been reported by Arends (1), who used a rotating sector photometer and discontinuous source. The curves are qualitatively alike, but differ in extinction peak. The value at maximum, measured from the published curve, is 1.59×10^4, 20 per cent lower than that found by us. This difference may be attributed in part to solvent effect.

Quantitative analysis of pure solutions of triphenylethylene.—With a knowledge of ϵ, quantitative measurements of concentrations may be made, with the simple Beer-Lambert relationship. In the conventional procedure the optical density of the unknown solution in a cell of known length is measured, preferably at the wave length of maximum absorption,[4] with a divided beam photometer, whence the concentration may be calculated. Since the optical density may be measured with an error of 3 to 10 per cent, the concentration is correspondingly accurate. At an optical density of approximately 1 (accuracy about 5 per cent) the concentration of triphenylethylene would be 0.625 mgm. per 100 cc. or 0.0062 mgm. per cc. With a convenient aliquot of 5 cc. in a 2 cm. cell approximately 0.03 mgm. would be measurable. At a density of 0.1, 0.003 mgm. could be measured with less accuracy.

Measurement with Baly tube and densitometer.—In the divided beam photometer the photographic emulsion is used as a null device for detecting equal blacknesses. With the advent of the photocell and densitometer it has been feasible to make absolute measurements of the transparency of a photographic emulsion. Accordingly it is possible, with a single beam of constant intensity and a densitometer, to measure the unknown concentration of a solution by graphical comparison with known concentrations on the same plate.[5] This procedure has been previously employed satisfactorily in the analysis of cocaine in tissue (22). The accuracy of transparency measurement by densitometer equals or betters the null method measurement of optical density; and in addition, a number of determinations may be made on a single plate. However, a series of known concentrations must be photographed for each plate.

With a Baly tube, with little loss of accuracy due to comparison of unequal path lengths, only one

[1] The other estrogens examined were estrone, estradiol, estradiol dipropionate, and diethylstilbestrol.

[2] We are indebted to Dr. J. A. Morrell, of E. R. Squibb and Sons, for the sample of triphenylethylene.

[3] The statement of half-width aids in the visualization of a simple symmetrical curve.

[4] It follows from Beer's law that optical density varies most rapidly with changing concentration at the wave length of maximum extinction.

[5] Also by using a sensitometric procedure (9), such as a step-sector, the response of an emulsion may be calibrated to reduce densitometric readings to corresponding intensity and density values. The absorption of vitamin E has been measured in this way (7). In general it is more clumsy and less accurate than the null method.

standard concentration will suffice. The unknown solution or solutions in a quartz Baly tube are spectrographed (Fig. 2b) at one or more path lengths,[6] followed by a similar series of spectrograms of a properly chosen standard concentration, 0.0005 per cent of triphenylethylene (Fig. 2c). Transparencies of the unknown and standard concentrations are measured at wave lengths of maximum absorption. From Fig. 3 (log transparency *vs.* path length) the transparency of the unknown may be interpolated and read in terms of a known path length of the standard concentration, whence, by application of Beer's law,

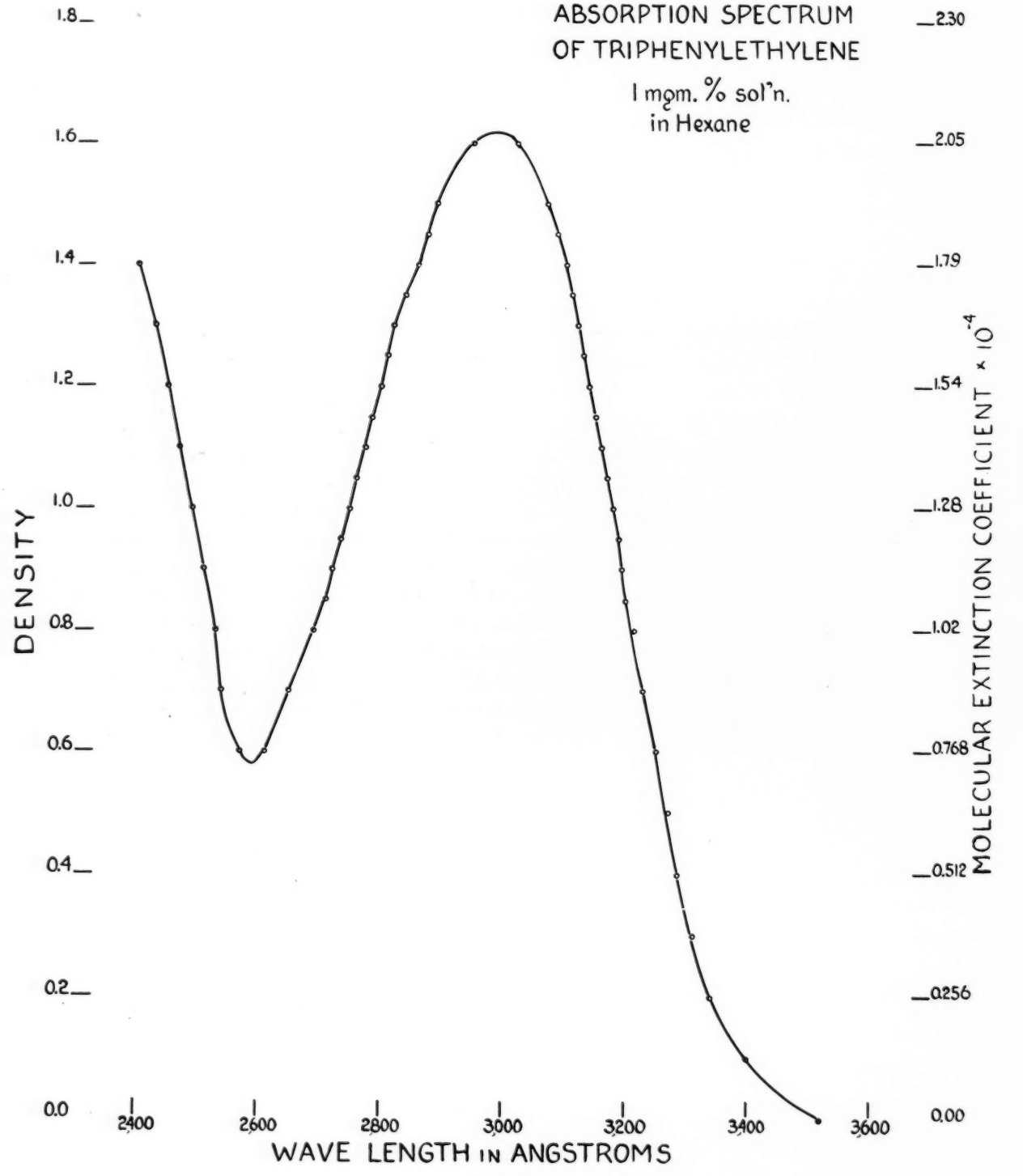

FIG. 1

[6] If a series of path lengths of the unknown is used a qualitative study of the absorption picture is simultaneously presented.

its concentration may be readily evaluated. The latitude of measurement is more restricted than in the null method because each spectrogram must be taken with constant source and constant exposure time. With the single beam method a cell of smaller aperture may be used and of correspondingly greater length for the same volume of solution, thus permitting higher sensitivity in measuring pure solutions. For example, a cell of 1 cc. volume and 35 mm. length permits the measurement of a lesser absolute amount by 1/9 (0.0007 mgm. at an optical density of one) as compared with the null method (*cf.* previous section).

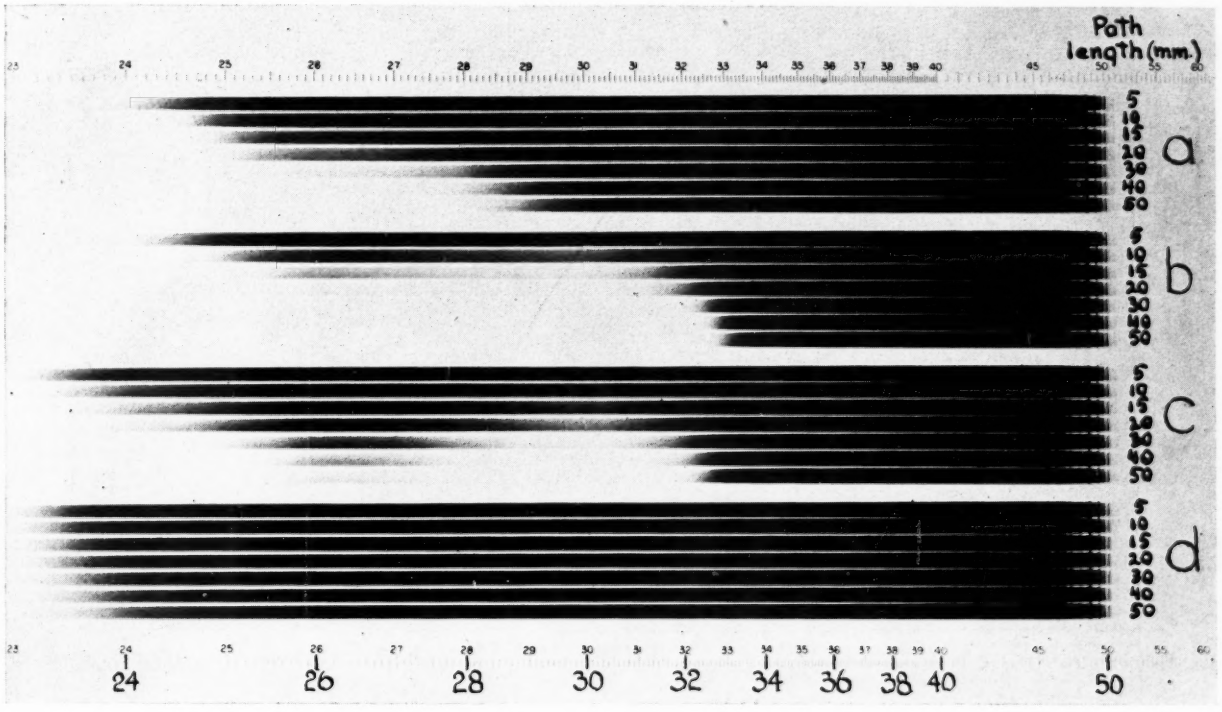

FIG. 2.—Illustrative Baly tube spectra of:

(a) Extract of 0.3 gm. control mamma.
(b) Extract of 0.3 gm. mamma containing triphenylethylene.
(c) 0.0005 per cent triphenylethylene (in octane).
(d) Solvent octane.

It is rarely the case that measurement is made of pure solutions in biological studies, but rather of impure extracts; accordingly maximum sensitivity cannot be attained.

ASSAY OF TISSUE EXTRACTS

Substances in tissue other than triphenylethylene that have ultraviolet absorption (background) at the same wave lengths must be removed or minimized before quantitative measurements can be attempted. The extraction procedure (Fig. 4) adopted for this purpose involved only differential solvent action (17) designed to remove "polar" compounds from the relatively "nonpolar" triphenylethylene. Radical chemical procedures such as hydrolysis were avoided as possible sources of loss.[7]

Extraction procedure.—After removal from the mouse the fresh tissues were minced with scissors, weighed, and then stored in acetone preparatory to extraction for the spectrochemical measurement of triphenylethylene according to the scheme described in Fig. 3.

The tissue (0.1 to 8.0 gm.) was removed from the storage container and floated in water for 5 minutes to replace sufficient water lost to the acetone to promote freezing. This water-containing tissue was then frozen in liquid air for 5 minutes and ground in a mortar chilled with solid CO_2.

The ground tissue plus the storage acetone was then heated for a few minutes on a water bath at 80° C. This served further to break down the tissue. After the acetone was evaporated, the powdered tissue was extracted with 60 cc. of ether in a Soxhlet apparatus for a period of 24 hours. The ether was then washed by allowing about 10 cc. of water to drip through the ether layer in a separatory funnel. The

[7] It was observed, for example, that triphenylethylene in pure solutions was stable to alcoholic saponification, but was decomposed by the same procedure if the triphenylethylene were added to tumor tissue *in vitro*.

washing reduced the tendency to form troublesome emulsions. The ether was then evaporated to dryness under a fan on a gently heated water bath. All traces of ether must be removed slowly without overheating, for this may result in the formation of resinous compounds with ultraviolet absorption, particularly if alkali is used in later stages. Two fractions were then extracted from the dried ether residue.[8] First the residue was extracted with 50 cc. of alcohol, U.S.P. XI, and then separated with 25 cc. of spectro-

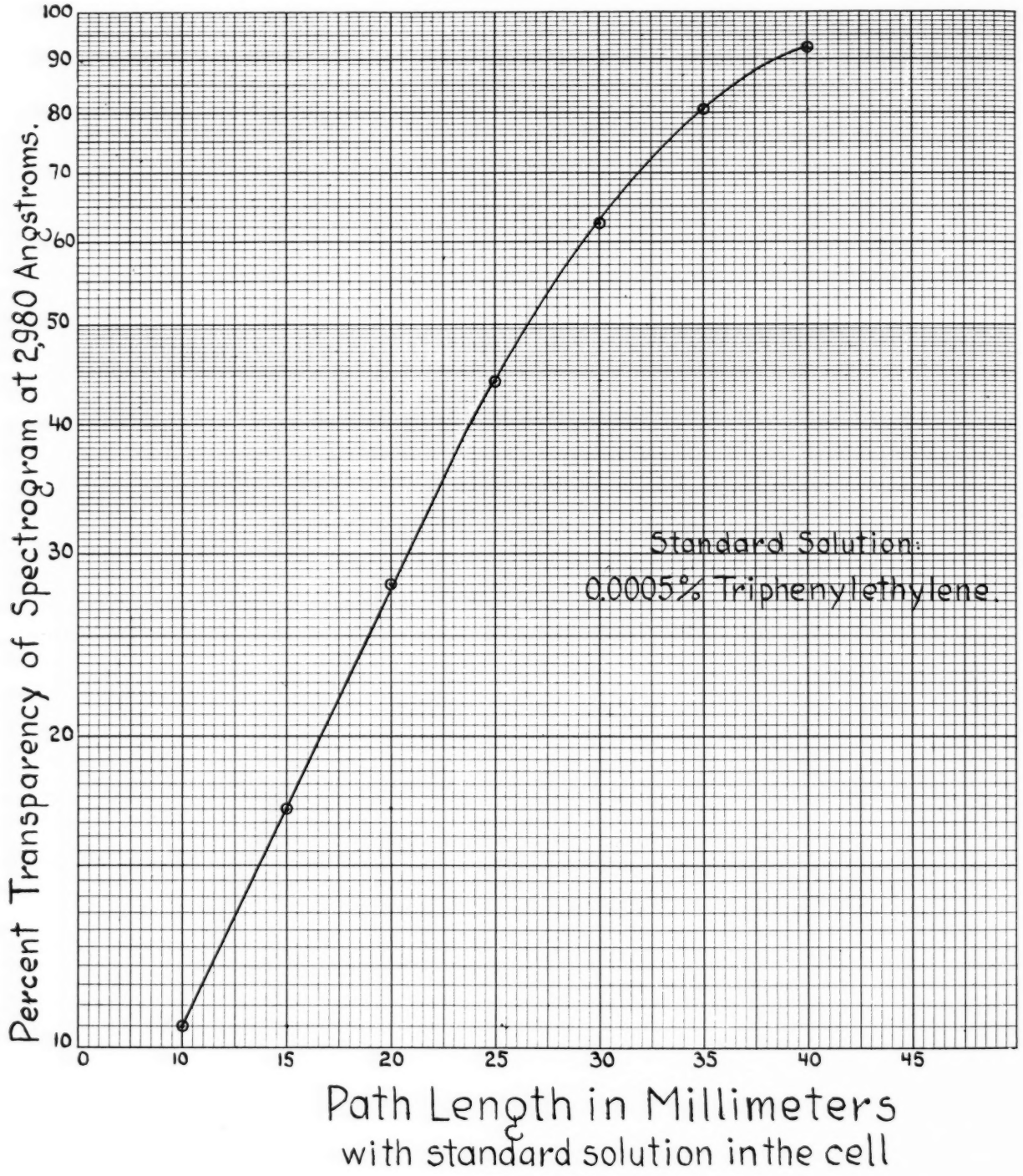

FIG. 3. Typical calibration curve for spectrophotometric assay of triphenylethylene.

Plate transparencies of the unknown in a cell of length l_x were measured with a densitometer at the wave length of maximum absorption of the estrogen. From the calibration curve the equivalent path length, l, of a 0.0005 per cent solution, c, may be determined. Applying Beer's law the concentration, c_x, of the unknown solution may be evaluated because: $c_x l_x = cl$.

[8] Adsorption with kaolin, permutit, and charcoal were unsatisfactory because of loss of estrogen.

graphically pure octane.[9] The "alcohol-soluble" fraction, which contains the triphenylethylene, was extracted with three 15 cc. portions of octane for complete recovery. The octane extract was then centrifuged to remove the last traces of alcohol and brought up to final volume of 50 cc. for spectrographic examination.

The extraction procedure does not eliminate substances similar in solubility to triphenylethylene. An

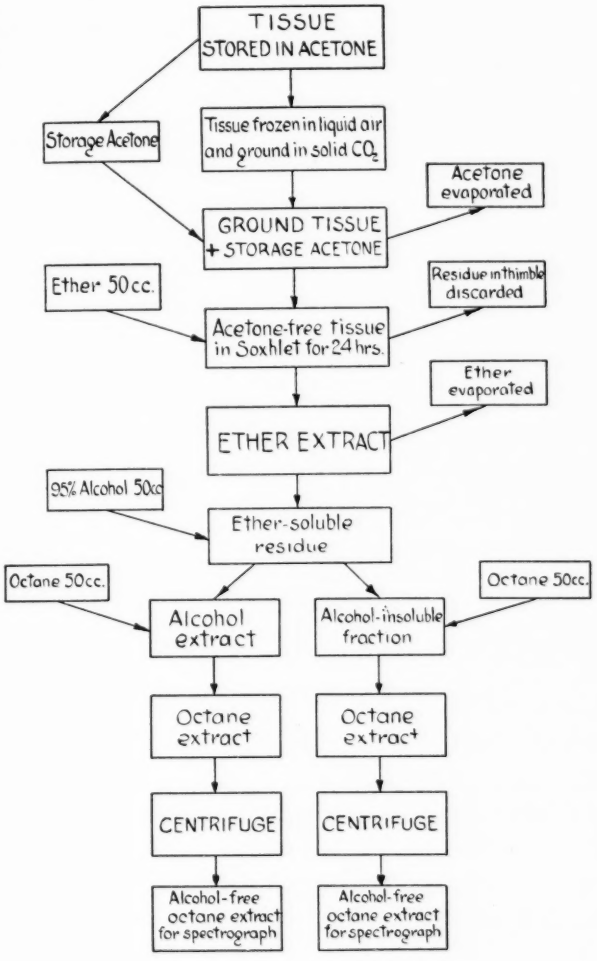

FIG. 4.—Steps in the extraction of tissues (mammary gland, tumor, etc.) for spectrographic assay of triphenylethylene and incidentally for detection of related metabolites.

appreciable background absorption remained that reduced the minimum amount of triphenylethylene detectable about tenfold as compared with pure solutions of triphenylethylene. However, the sensitivity was sufficient for our purposes and the background absorption was of additional interest.

[9] We are indebted to Dr. L. V. Steck, of the Shell Development Company, Emeryville, California, for concomitant "octanes" purified by a conventional method (24). The purified octane boiled at 112-114° C. and probably consisted of dimethylhexane and various trimethylpentanes (25).

MEASUREMENT OF TRIPHENYLETHYLENE IN TISSUES

Measurement of triphenylethylene added to tumor tissue in vitro.—The background absorption of tumor tissue was originally determined in samples of a spontaneous tumor, rat sarcoma 180. The background absorption of the estrogen-induced tumors was similar (compare Fig. 5, a and b). When the extract of approximately 4 gm. of tumor was taken up in 50 cc. of octane, the background at 3,000 Å in a 30 mm. path length did not significantly interfere with the measurement of triphenylethylene. Upon the addition of 1 mgm. of triphenylethylene to 1 gm. of tumor, 92 per cent was recovered. That the background was negligible was verified by observing that the yields from measurements at three different path lengths differed by only 3 per cent. Essentially this means that these extracts obey Beer's law, and therefore are effectively pure solutions. By calculation, as little as 0.05 mgm. of triphenylethylene per gm. of tumor tissue would be measurable and 0.025 mgm. would be qualitatively discernible.

Measurement of triphenylethylene in mamma of treated mice.—A number of control mammary tissue samples were studied to determine the nature of their background absorption. The mammae of 16 untreated spayed female mice of a nontumor strain (Palmer) were pooled and examined in 8 aliquot parts of 0.5 gm. each. These samples were extracted independently, extreme variations in time and temperature that might occur in the extraction procedure being introduced. The background was found to be nearly constant from sample to sample. The residual impurities in mammary tissue extracts had definitely more background per gram than tumor tissue (compare Fig. 5, d and b), a difference that is interpreted as due to the presence of fatty substances in the mamma and not in the tumor. The background of untreated mammary tissue samples from C3H male mice was compared with that of untreated mamma from mice of the Palmer strain. No differences in background were evident between sexes and strains. In connection with another investigation (2) 8 independent extracts of lactating mammae from untreated females of spontaneous tumor strains (A, C3H, dba) and 3 nontumor strain (C57) mice showed no strain differences spectrographically. The lactating glands have significantly less background absorption per unit weight than nonlactating resting ones (Fig. 5, e and d). It is possible that this is due to milk content and to the increased ratio of mamma to supporting fat.

The efficiency of recovery of triphenylethylene from mammary tissue was determined *in vitro* by adding 0.5 mgm. of triphenylethylene to 0.3 gm. of freshly excised mammary tissue (Fig. 2). In four independent

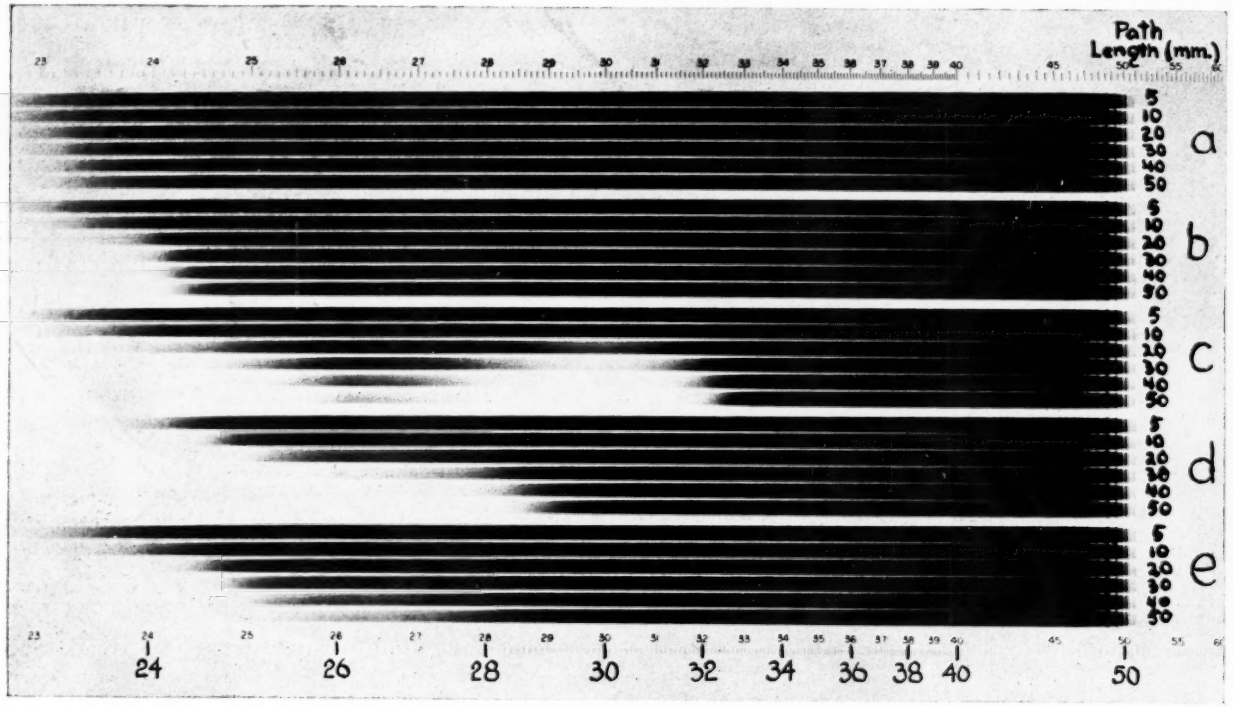

FIG. 5.—Illustrative spectra of:

(a) Solvent: The solvent is transparent at all wave lengths within the limit of sensitivity of the emulsion.

(b) Extract of 0.3 gm. of mammary cancer tissue. The cancer tissue extract is little different in transparency from the solvent.

(c) 0.0005 per cent triphenylethylene (in octane).

(d) Extract of 0.3 gm. of control mammary tissue. There is a definite increase in absorption compared with b.

(e) Extract of 0.3 gm. of lactating mamma. Less absorption than control mammary tissue.

experiments the recovery of triphenylethylene was uniformly high, averaging 97.5 per cent, with a probable error of ± 2.7 per cent.

Other tissues.—Preliminary studies of the background absorption of pancreas, kidney, liver, and uterus from triphenylethylene-treated animals indicate that this method is applicable also to these organs.

SUMMARY

1. The absorption spectrum of triphenylethylene has been measured with a Spekker photometer.

2. A spectrochemical method for the assay and study of triphenylethylene (α-phenylstilbene) in tissues affected by this estrogen is described, inclusive of a quantitative method of analysis with a single beam, Baly tube, and densitometer.

3. A chemical extraction procedure to minimize background absorption is described.

4. The sensitivity of the method for mammary and other tissues is determined to be adequate for biological studies.

REFERENCES

1. ARENDS, B. Über Licht-Absorption und Komplexbindung bei ungesättigten Verbindung. Ber. d. deutsch. chem. Gesellsch., **64**:1936-1951. 1931.

2. DEOME, K. B., STRAIT, L. A., and MCCAWLEY, E. L. The Ultraviolet Spectrographic Examination of the Fat Fraction of Mouse Milk and Mammary Glands. Science, **96**: 301-302. 1942.

3. DIETERT, H. W., and SCHUCH, J. A New Projection Comparator-Densitometer. J. Optic. Soc. America, **31**:54-57. 1941.

4. DOBRINER, K., GORDON, E., RHOADS, C. P., LIEBERMAN, S., and FIESER, L. F. Steroid Hormone Excretion by Normal and Pathological Individuals. Science, **95**:534-536. 1942.

5. DOBRINER, K., LAVIN, G. I., and RHOADS, C. P. The Spectroscopic Study of Biological Extracts. I. Urine. Cancer Research, **2**:79-94. 1942.

6. DOBRINER, K., RHOADS, C. P., and LAVIN, G. I. The Spectroscopic Study of Biological Extracts. II. Detection, Isolation, and Biological Effects of the Metabolites of 1,2,5,6-Dibenzanthracene. Cancer Research, **2**:95-107. 1942.

7. EVANS, H. M., EMERSON, O. H., and EMERSON, G. A. The Isolation from Wheat Germ Oil of an Alcohol, α-Tocopherol, Having the Properties of Vitamin E. J. Biol. Chem., **113**:319-332. 1936.

8. FRANK, R. T., GOLDBERGER, M. A., SALMON, U. J., and FRIEDMAN, R. Significance of Estrogenic Factors in Tumors and Tissues of the Human Female. Proc. Soc. Exper. Biol. & Med., **32**:1665. 1935.

9. HARRISON, G. Current Advances in Photographic Photometry. J. Optic. Soc. America, **24**:59-72. 1934.

10. HELL, C., and WIEGANDT, F. Über Verbindungen der Stilbenreihe. Ber. d. deutsch. chem. Gesellsch., **37**:1429-1432. 1904.

11. Hieger, I. The Spectra of Cancer-Producing Tars and Oils and of Related Substances. Biochem. J., **24**:505-511. 1930.
12. Jones, R. N. Further Observations on the Absorption Spectra of Derivatives of 1,2-Benzanthracene. J. Am. Chem. Soc., **63**:151-155. 1941.
13. Jones, R. N. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. I. Introduction. Cancer Research, **2**:237-244. 1942.
14. Jones, R. N. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. II. Determination of 1,2,5,6-Dibenzanthracene and 4′,8′-Dihydroxy-1,2,5,6-Dibenzanthracene in Rat Excreta. Cancer Research, **2**:245-251. 1942.
15. Jones, R. N. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. III. Distribution of 1,2,5,6-Dibenzanthracene in Rats Following Subcutaneous Injection in Olive Oil. Cancer Research, **2**:252-255. 1942.
16. Lewis, D., and Geschickter, C. F. The Demonstration of Hormones in Tumors. Ann. Surg., **104**:787-797. 1936.
17. Lorenz, E., and Shear, M. J. Studies in Carcinogenesis. II. The Detection of Dibenzanthracene in Mouse Tumors Induced by This Hydrocarbon. Am. J. Cancer, **26**:333-343. 1936.
18. Lorenz, E., and Stewart, H. L. Intestinal Carcinoma and Other Lesions in Mice Following Oral Administration of 1,2,5,6-Dibenzanthracene and 20-Methylcholanthrene. J. Nat. Cancer Inst., **1**:17-40. 1940.
19. Mohs, F. E. Lack of Estrin Concentration in Adenofibroma of the Mammary Gland in Rats. Am. J. Cancer, **29**:356-362. 1937.
20. Nathanson, I. T. Sex Hormone Excretion in Neoplastic Disease. Cancer Research, **2**:727-728. 1942.
21. Pincus, G. Steroid Excretion in Cancerous and Noncancerous Persons. Cancer Research, **2**:729. 1942.
22. Strait, L. S., Aird, R. B., and Weiss, S. A Method for the Rapid Isolation and Spectrographic Measurement of Cocaine from Brain Tissue. J. Pharmacol. & Exper. Therap., **75**:363-374. 1941.
23. Strait, L. S. Assay of Organic Molecules in Tissue from Absorption Spectra Using a Baly Tube and Densitometer. Physical Rev., **62**:304. 1942.
24. Twyman, F., and Allsopp, C. B. The Practice of Absorption Spectrophotometry. London: A. Hilger, Ltd. 1934, p. 67.
25. Ward, A. L., and Kurtz, S. S. Refraction, Dispersion and Related Properties of Pure Hydrocarbons. Indust. & Engin. Chem. (Analytical Ed.), **10**:559-576. 1938.
26. Winterstein, A., and Vetter, H. Fraktionierung und Reindarstellung organischer Substanzen nach dem Prinzip der chomatographischen Adsorptionanalyse. VI. 1,2-Benzpyren. Ztschr. f. physiol. Chem., **230**:169-174. 1934.

A Spectrochemical Study of Estrogen-Induced Mammary Cancer in Mice*

II. Distribution of Triphenylethylene in the Mamma and in Mammary Cancer Induced by This Estrogen

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INTRODUCTION

The induction of mammary cancer in mice following the protracted administration of estrogens, both naturally derived and synthetic, has been well established (3). This method of producing mammary cancers offers advantages in a study of the role of estrogens in carcinogenesis: Not only are the cancers a result of one single additional factor, the estrogen, but also they appear at a site *remote* from that of application, which makes it unnecessary to account for any part of the agent not entering into carcinogenesis. This is not usually the case where tumors result from carcinogenic hydrocarbons. In fixing the role of an estrogen in carcinogenesis, time and place are important factors. Estrogenic substances disappear rapidly from the body whereas the induction of mammary cancer requires many months. Between these early and late manifestations the irreversible effects of the estrogen occur.

Attention has been devoted primarily to studying the late manifestation, the established cancer. Investigations on the presence and effect of estrogens in the mammary gland are few and limited to bioassays. The amount of estrogens in human breast cancer was reported by Lewis and Geschickter (5) to be approximately the same as that given by Frank and his associates (2) for normal skeletal muscle, and Mohs (6) found equivalent amounts of estrogen in estrogen-induced benign fibroadenomas of the mamma in rats and in skeletal muscle from the same animals.

Little attention has been given to a direct study of the relationship between the injected estrogen and mammary tissue at a time preceding tumor formation. After an estrogen-induced cancer arises in one mamma of a mouse, additional mammary cancers not uncommonly appear if the animal survives. It is reasonable to suppose that these chronically hyperplastic glands are precancerous.[1] Precancerous tissue may be assumed to be the site of those changes that initiate carcinogenesis and that may represent its crux. Because the role of the estrogens is obscure, the experiments reported here were designed to determine whether carcinogenic doses of an estrogen go to the mamma or to the induced mammary cancer, and if glands treated with single and multiple injections differ in the consumption of estrogen.

EXPERIMENTAL

Line-bred male and female C3H mice[2] of spontaneous mammary cancer strains and Palmer mice[3] were injected subcutaneously with a synthetic estrogen, triphenylethylene, until mammary cancer developed. Although female C3H mice spontaneously develop mammary tumors, males do not unless an estrogen is administered. The animals received 5 mgm. of triphenylethylene (10 times the estrogenic dose) in the smallest practical volume (0.05 cc.) of sesame oil by subcutaneous injections once a week. The injections were made in the back and the site was changed weekly through the four quadrants to minimize the formation of oil cysts. There may be variations in immediate absorption due to the variations in vascularity of the injection site and later from the formation of oil cysts. No oil or oil cysts were included in the

* This investigation was supported in part by a grant from the Charles and Blanche Wolf Foundation for Cancer Research. With the technical assistance of Mr. M. K. Hrenoff.

** International Cancer Research Foundation Fellow, 1942-1943.

[1] Precancerous mamma is used in this report synonymously with mamma repeatedly treated, to refer specifically to the remaining noncancerous hyperplastic mammary glands of mice treated with a series of estrogen injections until tumor has appeared in one or more of these organs.

[2] The mice used were obtained through the courtesy of Dr. K. B. DeOme, Department of Veterinary Science, University of California, Berkeley.

[3] These are from a colony of low spontaneous tumor strain mice maintained locally for 30 years.

dissected mamma. Triphenylethylene (α-phenylstilbene) was chosen because its intense spectrum and high effective dose are both favorable for tracing it in the tissue (8). A synthetic estrogen avoids confusion with the natural estrogens that may be present in the intact animal.

Carcinomas appeared between the 23rd and 60th week of treatment; at this time the total amount of estrogen administered amounted to 115 to 300 mgm. The diagnosis was confirmed by histological examination in every case. Induction time and incidence were in agreement with those reported by Robson and Bonser (7). The animals were sacrificed when the tumors were still small to reduce the variable effects of necrosis. In addition to the tumor, the remaining mammary glands, without visible neoplasm, and other tissues, were excised for spectroscopic examination.

Because it is known that the estrogens are rapidly removed from the body (1) the animals were sacrificed and the tissues excised at various predetermined intervals after the last dose. Time-variable data of this kind were chosen to yield information on the rate of disappearance of the estrogen from mamma and tumor.

Samples of mammary tissue with and without estrogen were exposed to loss of acetone (8) and to the effects of oxygen at 50° C. These exaggerated conditions of storage, which favored autolysis, produced no appreciable effect on the background absorption compared with freshly prepared samples and did not destroy the estrogen.

The details of the spectrophotometric method of analysis and preliminary chemical purification have been described (8). The preliminary purification by differential solvent extraction was devised to retain triphenylethylene and to minimize the background absorption from other constituents of the tissue. The method of analysis of the estrogen is sufficiently sensitive quantitatively to detect at least one-tenth (0.05 mgm.) of the estrogenic dose (0.5 mgm.), or approximately 1/2300 of the total minimal amount injected before mammary cancer appeared.

EXPERIMENTAL RESULTS

Tumor tissue.—Twenty-one samples of mammary carcinoma examined spectrochemically were found to contain *no* triphenylethylene (see Table I). If triphenylethylene were present at all it must have been in amounts less than the minimal quantity detectable, 0.025 mgm. per gm. The times of excision varied from 1 to 33 days after the last dose. No significant amount of background in the region of triphenylethylene absorption was observed in the mammary cancer extracts. For illustration, in Fig. 1 the spectogram (b) shows no appreciable absorption in the region at 3,000 Å of triphenylethylene absorption as in (e). Comparison of the extracts in (b) and (c) of Fig. 1 shows that tumor, although some 7 times the weight of the control mammary tissue, had less background absorption. The spectra were uniform in appearance and not significantly different from those of spontaneous C3H mammary cancers.

Mamma repeatedly treated.—In comparison with the tumors, 24 corresponding samples of mammary tissue repeatedly injected were examined in the same manner. These precancerous tissues from mice with triphenylethylene-induced tumors had been under the influence of multiple injections of triphenylethylene but did not appear cancerous upon gross examination. In contrast with the tumors from the same animals these mammary glands were found to contain triphenylethylene in a uniform manner. It is evident from Table II that estrogen appearing in precancerous mammae as a result of the last injection disappears within 7 days at most but significantly large amounts of estrogen (0.2 to 2.0 mgm. per gm.) are found for at least the first 3 days in all cases examined. Observe, for example, that a precancerous mammary extract Fig. 1, d, in contrast with the control mammary extract 1, c, has an absorption band of triphenylethylene corresponding with that of pure triphenylethylene (e). From these data and from Fig. 4 it may be inferred that at the time of each successive weekly treatment the estrogen from the previous injection had been eliminated from the precancerous mamma.

Pancreas.—The pancreas was selected as a control organ for mamma in the triphenylethylene-treated mice because it also is a glandular structure in a fatty matrix. Although it thus offers about the same physical characteristics as mamma it does not respond physiologically to estrogen.

Eight samples of pancreas from the mice for which mamma data are reported (Table II) were analyzed. The intervals after final injection were as follows: 1, 2, 3, 9, 12, 22, and 32 days. The triphenylethylene content of the pancreas with regard to time interval was parallel with that found in the corresponding mammary glands. Traces of estrogen were found in the first 3 days (Fig. 2) and none in the remaining intervals. The comparative concentration in the pancreas appeared to be less than in the mamma. To obtain more than semiquantitative data for the relatively smaller pancreatic samples either improvements in the extraction procedure must be made or pooled samples of 5 or more mice must be taken. Additional studies are in progress.

New absorption spectra.—Although triphenylethylene had disappeared from the precancerous mamma by 7 days after the last injection, absorption back-

TABLE I: ANALYSIS OF MAMMARY TUMORS FOR TRIPHENYLETHYLENE

Time interval between last dose and excision of tissue, days	1	2	3	4	7	9	10	12	18	21	22	24	25	26	29	32	33	43
Triphenylethylene found, mgm. per gm. of tissue	None *	None	None	None	None	None	None *	None *	None †	None †	None †	None †	None		None	None †	None *	None
		None	None		None										None			
					None													
Total amount of triphenylethylene administered, mgm.	355 *	205	324	127	114	108	115 *	147 *	174 †	171 †	120 †	130 †	189		290	184 †	165 *	115
		217	327		167										127			
					167													
Weight of tissue analyzed, gm.	1.06 *	0.73	0.20	2.8	2.9	0.97	1.6 *	2.2 *	4.3 †	3.8 †	1.8 †	2.1 †	3.6		2.2	2.0 †	4.0 * †	1.37
		1.4	1.6		2.6										1.2			
					0.62													

In Tables I and II C3H male mice were used except where otherwise noted. All animals received 5 mgm. of triphenylethylene once a week in 0.05 cc. sesame oil. The injections were made in the subcutaneous tissue of the sacral region of the back until tumor of mamma appeared (20 or more doses). The tumor and remaining precancerous mammary tissue were removed and stored in acetone. The tissues and storage acetone were extracted and the amount of triphenylethylene determined spectrographically as described in Paper I (8).

* Animal was a female mouse of the Palmer strain.

† Extraction involved saponification.

TABLE II: ANALYSIS OF PRECANCEROUS MAMMA FOR TRIPHENYLETHYLENE

Time interval between last dose and excision of tissue, days	1	2	3	4	7	9	10	12	18	21	22	24	25	26	29	32	33	43
Triphenylethylene found, mgm. per gm. of tissue	0.30 *	2.0	0.89		None		None *	None *	None	None	None	None	None	None	None	None	None *	None
	0.35 *		1.4		None					None					None			
	1.1 *		0.55												None			
Total amount of triphenylethylene administered, mgm.	355 *	205	324		114		115 *	147 *	174	155	120	130	189	175	290	184	165 *	115
	377 *		327		167					171					290			
	355 *		319												127			
															309			
Weight of tissue excised and analyzed, gm.	0.88 *	0.84	0.21		0.31		0.25 *	0.30 *	1.7	0.66	1.0	1.1	1.0	0.2	0.49	0.30	1.0 *	1.3
	0.42 *		0.42		0.05					0.88					0.20			
			0.57												0.52			
															0.47			

Those mammary glands in which "new" bands were found are underlined.

* Animal was a female mouse of the Palmer strain.

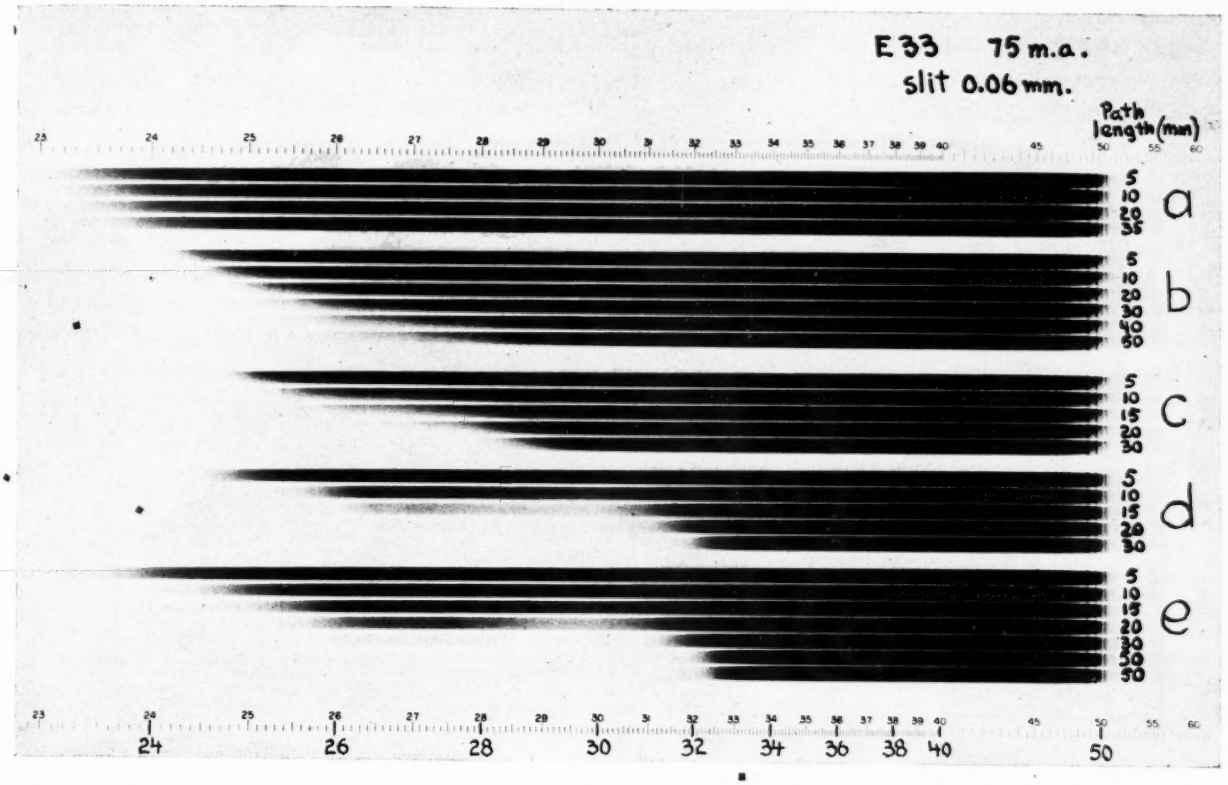

FIG. 1.—Illustrative spectra. Comparison of spectra of estrogen-induced mammary cancer with companion precancerous mamma and control mamma. Spectrum of:

(a) Solvent.
(b) Extract of 3.6 gm. of mammary cancer.
(c) Extract of 0.5 gm. of control mamma.
(d) Extract of 0.35 gm. of precancerous mamma excised 1 day after last administered dose of triphenylethylene.
(e) 0.0005 per cent standard solution of triphenylethylene.

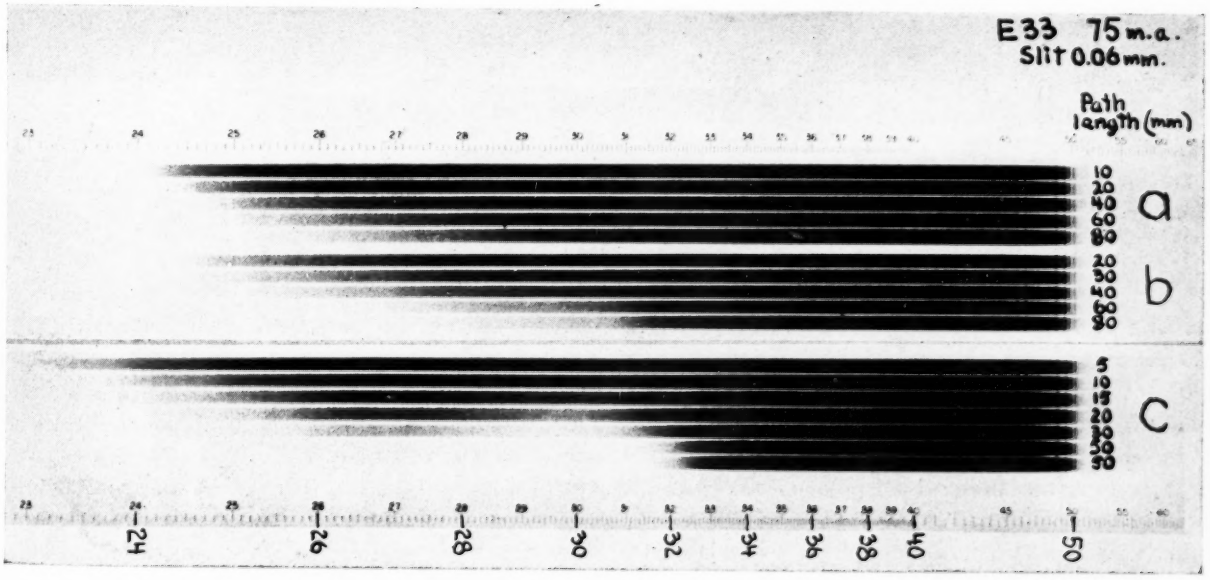

FIG. 2.—Illustrative spectra. Triphenylethylene in pancreas. Spectrum of:

(a) Extract from 0.11 gm. of control pancreas.
(b) Extract from 0.17 gm. of pancreas excised from mouse given a series of injections, 1 day after last administered dose. Compare with Fig. 1, d.
(c) 0.0005 per cent triphenylethylene.

ground in variable amounts appeared. Compare (a) with (b), (c), and (d) in Fig. 3. The amount of absorption seemed greater than that found (8) in control untreated mammary gland or tumor. For example, in Fig. 3, a and d, it may be seen that 0.3 gm. of precancerous mamma, removed 12 days after the last injection, had significantly more absorption background than the 0.5 gm. of untreated control mammary sample. In 5 cases of 18 observed between 12 and 43 days after the last injection there were characteristic features to the absorption background, in correspondence with the ones given mutiple injections. Triphenylethylene was present from 6 to 24 hours after injection in the mamma of females given one treatment and was not present 2 and 3 days after injection. The mammary glands of male mice appeared to contain more triphenylethylene at corresponding times than did those of the females (Fig. 4, Paper I). The apparent difference in absorption of male and female mamma is under further study and will be reported in a later communication. Spayed mice were used in some single injection experiments in order to

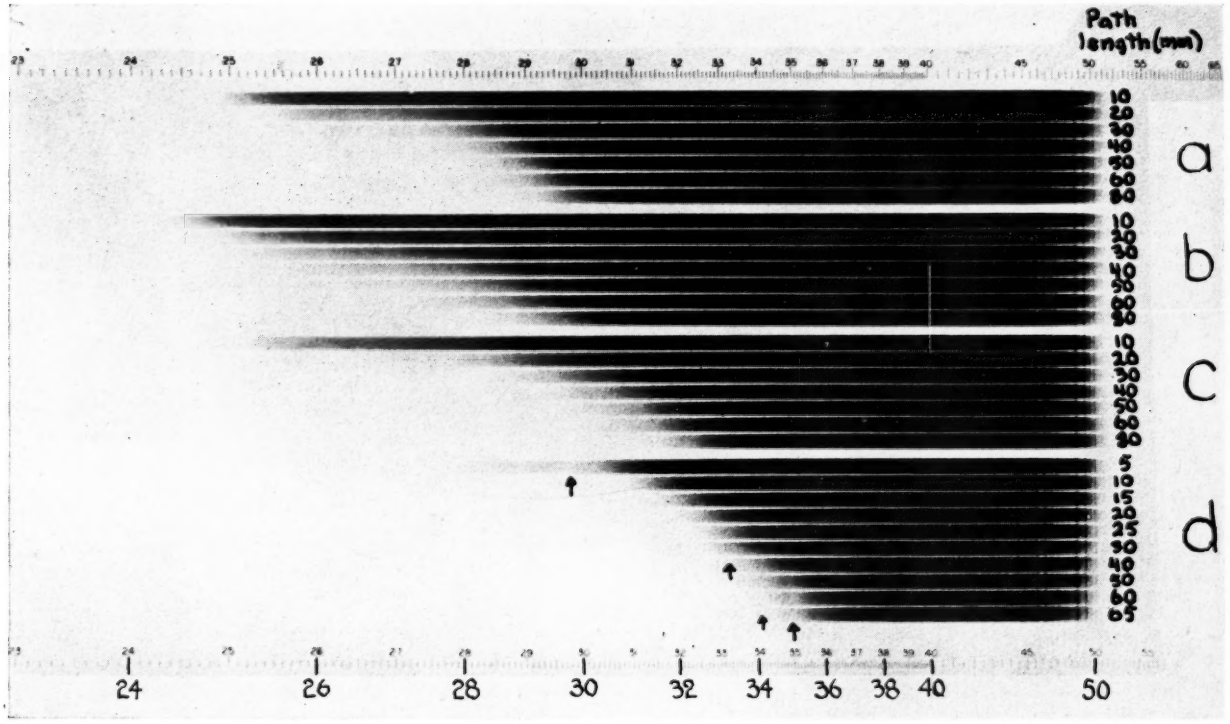

FIG. 3.—Illustrative spectra. Various stages of residual absorption in precancerous mammae after the disappearance of triphenylethylene. Spectrum of:

(a) Extract from 0.5 gm. of control mamma.
(b) Extract from 0.25 gm. of precancerous mamma.
(c) Extract from 0.49 gm. of precancerous mamma.
(d) Extract from 0.3 gm. of precancerous mamma.

consisting of faint, sharp bands at 3,500 Å, 3,400 Å, 3,225 Å, and a broader band of higher extinction at 3,000 Å (Fig. 3, d). The 5 cases are underlined in Table II. The "new" absorption spectrum found in the mamma after treatment with triphenylethylene may represent the presence of a substance possibly related to the role of the estrogen in carcinogenesis.

Mammary tissue after single injection.—To compare the utilization of triphenylethylene by precancerous and by previously untreated mamma, control mice were given one single 5 mgm. dose of triphenylethylene. The animals were sacrificed at intervals between 5 and 24 hours and at 2 and 3 days after injection, verify, by vaginal smear, that the animals had absorbed and responded to the estrogen. Fig. 4 depicts schematically the alteration in the rate of elimination of the estrogen by singly and repeatedly treated or precancerous mammae. A comparison of the amount of triphenylethylene in mice given single and multiple injections indicates that the precancerous mamma contains more triphenylethylene and retains it longer than does the mamma after a single treatment. A direct comparison of the rates of elimination of estrogen after single and multiple treatments is justified because of the rapid elimination of each successive weekly dose by the repeatedly treated mamma. Each of these

doses is eliminated from the mamma after a series of injections in less than 7 days (Table II and Fig. 4).

That the altered ability of precancerous mammary tissue to retain estrogen is not attributable to the condition of hyperplasia alone has been verified by giving a single 5 mgm. dose to untreated mice with physiologically hyperplastic mammary glands. No triphenylethylene was found at 3 days in the case of a mouse just delivered of a litter; likewise in the case of a pregnant mouse at term no triphenylethylene was found 67 hours after the injection.

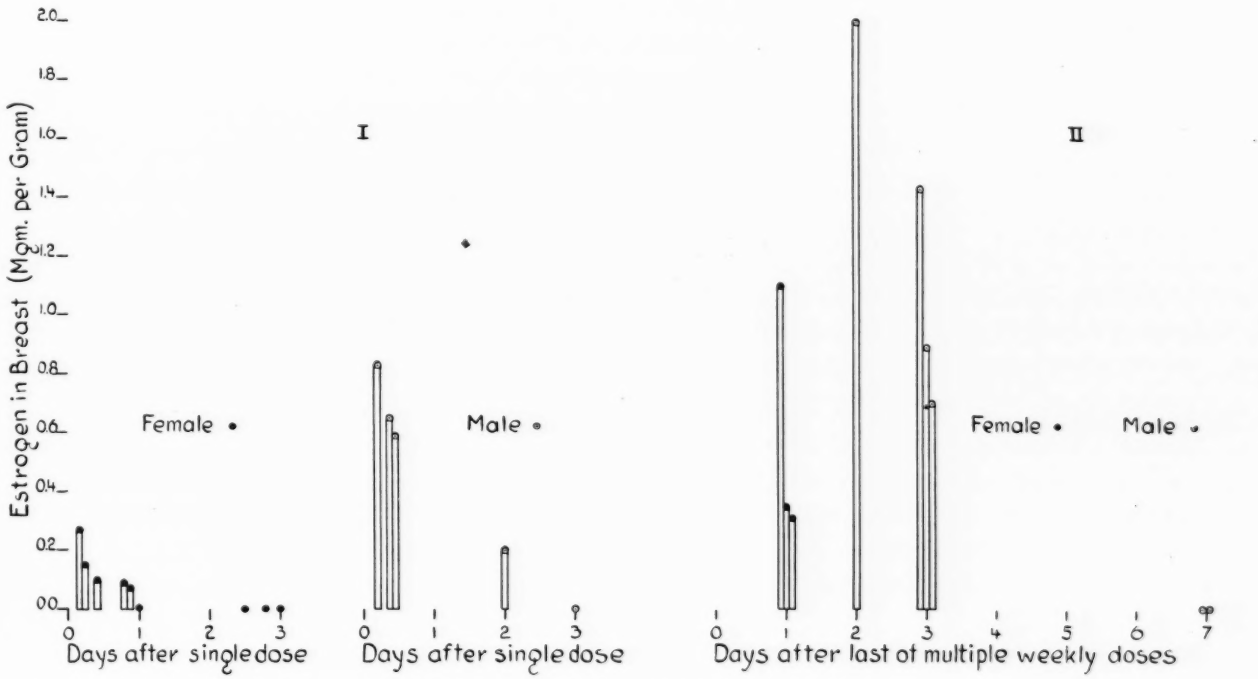

FIG. 4.—Comparison of rates of absorption of triphenylethlyene by precancerous and "resting" mammae.

(I) Absorption of estrogen by previously untreated ("resting") mammary gland.
(II) Absorption of estrogen by repeatedly treated precancerous mammary gland.

Triphenylethylene is not found in the mamma of female mice 1 day or in male mice 3 days after a single dose, whereas it is present in relatively large amounts when the single dose is the last of many, inducing the precancerous state. The absorption of estrogen by the mammae of male mice appears to be enhanced compared with that of females.

DISCUSSION

The uniform finding of no estrogen in estrogen-induced mammary carcinoma makes it appear likely that mammary cancer does not absorb or utilize the estrogen, triphenylethylene. Estrogens are proliferative stimuli to normal mammary tissue and are present in the proliferating gland. The absence of estrogen in mammary cancer induced by estrogen indicates that the significant effect of the estrogen takes place before the neoplasm is established.

Gilmour (4) has reported that transplanted mammary cancer grew no better in estrone-treated mice than in normal controls and concluded, "Estrone, therefore, does not exert any stimulating effect upon the rate of growth of the already malignant cell even when that cell was derived from the mammae." Although our observation concurs with Gilmour's conclusion, the observed disappearance of estrogen from the mamma within 3 days after administration (Fig. 4) makes it dubious whether the tumors she transplanted into estrogenized mice remained subject to the influence of the estrogen for more than a small fraction of the period of tumor growth. It would be desirable to extend Gilmour's experiments by continuing estrogen treatment after the transplantation and during the growth of the transplanted tumor.

In contrast with the absence of estrogen in the tumors the positive findings in mamma treated by single and multiple injections may signify changes in normal mammary tissue preceding the formation of cancer. The observation that precancerous mammae after a series of treatments contain the estrogen in larger amounts and retain it for longer periods of time than do the glands after single treatments shows that the precancerous mamma differs from the "resting" mamma and from mammary cancer in the utilization of the estrogen, triphenylethylene. The measurable difference in the rate of disappearance of the estrogen from repeatedly treated as compared with "resting" mamma may be an objective criterion of the precancerous state. Whether this change in "resting"

mamma as a result of prolonged treatment takes place suddenly, as in a mutation, or gradually may be determined by extending these studies to include similar measurements on the retention of estrogen by the mammary gland in the intermediate successive weekly periods between the first weekly dose and the appearance of tumor.

The changing ability of the mamma to accumulate estrogen may be connected with the initiation of malignancy. Study of this measurable property of estrogen content may accordingly serve to clarify the mechanism of the genesis of estrogen-induced mammary cancer.

Perhaps as significant as the data obtained is the adaptation of spectrochemical methods to estrogenic hydrocarbons in studying changes in small samples of tissue during the process of carcinogenesis in that tissue. Further investigations of the utilization of triphenylethylene in the mammary gland, liver, prostate, and particularly the uterus, are to be desired.

SUMMARY

By employing the absorption spectrum, the estrogen triphenylethylene can be detected in mammary tissue in amounts that are of the order of one-tenth of the estrogenic dose and 1/2300 of the minimal administered dosage producing mammary cancer.

As a result of the study of the tissues of 22 mice with cancer of the mamma resulting from repeated treatment with triphenylethylene, four significant observations have been made.

Triphenylethylene was not found in the cancer itself regardless of the state of development of the tumor or the time it was excised after administration of the last dose of triphenylethylene.

Although triphenylethylene was not evident in the cancer, it was present in appreciable amount in the remaining hyperplastic precancerous mammary tissue of the same animal if this was excised within 3 days following the last dose. It was not present in that excised after 7 days.

The estrogen was not observed in previously untreated mamma 2 to 3 days after a single estrogenic dose, while the mice were still in estrus.

Although extracts of the tumors studied exhibited without exception a characterless absorption spectrum, the hyperplastic precancerous mamma from these animals exhibited characteristic "new" absorption bands in 5 of 18 cases. Further investigation is necessary to determine the origin and significance of these bands.

These observations suggest that the mammary cancer induced by triphenylethylene does not absorb and retain triphenylethylene as does the hyperplastic precancerous mamma. The estrogen seems rather to play a direct role in carcinogenesis preceding the development of the cancer. The apparent decreased ability of the mamma after a series of treatments to eliminate estrogen as compared with that subjected to a single dose of the estrogen may be an objective criterion of the precancerous state.

REFERENCES

1. Dingemanse, E., and Lacquer, E. On the Inactivation of Estrone, Estradiol, and Their Monobenzoates in the Organism. Am. J. Obst. & Gynec., **33**:1000-1009. 1937.
2. Frank, R. T., Goldberger, M. A., Salmon, U. J., and Friedman, R. Significance of Estrogenic Factors in Tumors and Tissues of the Human Female. Proc. Soc. Exper. Biol. & Med., **32**:1665. 1935.
3. Gardner, W. U. Estrogens in Carcinogenesis. Arch. Path., **27**:138-170. 1939.
4. Gilmour, M. D. An Investigation into the Influence of Oestrone on the Growth and on the Genesis of Malignant Cells. J. Path. & Bact., **45**:179-188. 1937.
5. Lewis, D., and Geschickter, C. F. The Demonstration of Hormones in Tumors. Ann. Surg., **104**:787-797. 1936.
6. Mohs, F. E. Lack of Estrin Concentration in Adenofibroma of the Mammary Gland in Rats. Am. J. Cancer, **29**: 356-362. 1937.
7. Robson, J. M., and Bonser, G. M. Production of Mammary Carcinomas in Mice of a Susceptible Strain by the Synthetic Oestrogen, Triphenyl Ethylene. Nature, **142**:836. 1938.
8. Strait, L. A., McCawley, E. L., and Perry, I. H. A Spectrochemical Study of Estrogen-Induced Mammary Cancer in Mice. I. Chemical Preparation of Tissue and Analysis by Spectrophotometry. Cancer Research, **3**:370-377. 1943.

The Incidence of a Carcinogenic Factor in the Livers of Cancer, Noncancer, Cirrhotic, and Negro Patients*†

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INTRODUCTION

This is a report of a survey on the incidence of carcinogenic activity in extracts of various kinds of human livers. It is based on extracts from 67 cases tested in 896 mice.

Carcinogenic activity in extracts of human liver has now been reported by Schabad (11); Kleinenberg, Neufach, and Schabad (7, 8); des Ligneris (4); Hieger (6); Steiner (13, 15); and Sannié, Truhaut, and Guérin (10); and has failed of confirmation only by Gummel (5).

Despite all these papers it is not known at the present time what percentage of human livers contains this carcinogen, or with what types of tumors it is associated, although a few data are available on the latter point. For example, Schabad found that the extract of a liver from a patient with carcinoma of the stomach was carcinogenic, and Hieger stated that some of his tumors were induced by extracts of livers from persons who had lymphosarcoma. The other papers mentioned above gave the results either on extracts obtained from pooled livers, on extracts pooled before testing, or on animals injected with extracts from more than one liver.

Des Ligneris and Steiner both reported that extracts of livers from persons who had no neoplasm also produced tumors at the site of injection. The former tested Bantus in South Africa, and the latter white persons in America. Again, the carcinogenic activity cannot be correlated with the major disease diagnosis because the livers were pooled for extraction, and for the same reason it is impossible to know what percentage of the livers contained the carcinogen.

In order to establish the rôle, if any, of this liver carcinogen in the causation of human cancer it is important to know whether it is present in every liver or only in some, whether it is found in all persons who had a tumor, and whether it is associated with special types of tumors, or with tumors in certain locations as, for example, in the region from which portal blood drains to the liver. Could it be a chemical which is absorbed from the intestine, or carried from a tumor or a precancerous site to the liver? Correlation of its presence with the sex of the patients is important because most of the extracts previously reported to be carcinogenic almost certainly, because of the chemical methods used, contained steroid sex hormones. The relation of the presence or absence of carcinogenic activity in the livers to the age of the patients also might be important in view of the age incidence of malignant tumors. Does the amount of this carcinogen increase with age?

A survey of cirrhotic livers for carcinogenic factors was of special interest because of the frequency with which cirrhosis precedes primary carcinoma of the liver. For example, cirrhosis was present in 11 of 20 livers showing primary carcinoma, not excluding those that arose from the intrahepatic bile ducts, examined by members of this department, while, in contrast, cirrhosis, and that of a slight degree, was present in only 2 out of 21 cases in which a primary carcinoma arose in the gall bladder. Furthermore, in parts of the world where primary carcinoma of the liver is the commonest malignant tumor found at autopsy, hepatitis and cirrhosis are stated frequently to precede the tumor (1, 3). Des Ligneris (4) demonstrated carcinogenic activity in extracts from such noncancer livers. Is cirrhosis of the liver to be regarded as a precancerous lesion? Is there a carcinogen in some human livers that causes hepatitis, cirrhosis, and, in some cases, primary carcinoma?

Still another problem presents itself in relation to the Negro. In East and South Africa carcinoma of the liver is the commonest tumor in the Bantu races (2). Some American Negroes are probably of Bantu stocks (9). The incidence of primary carcinoma of the liver in the American Negro, however, appears to be

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much lower than in the African Bantu, and to resemble that of white persons in America. For example, in this department, autopsies on 4,856 white persons over 1 year of age disclosed primary carcinoma of the liver in 18 (0.37 per cent), while during the same period of time and in the same age group primary carcinomas of the liver were found in 2 Negroes among a total of 333 examined (0.60 per cent). If one accepts the statement that some American Negroes are of Bantu stocks, then this remarkable change in incidence of a malignant tumor assumes great importance. It can be explained theoretically on either a constitutional or on an environmental basis. Such a sudden change in the incidence of a primary tumor, following a crossing of the Atlantic Ocean, can probably be attributed to environmental factors. Des Ligneris has demonstrated carcinogenic activity in extracts of noncancerous Bantu livers. It would be of interest to know if this carcinogen is still present in the Afro-American. Is there loss of this liver carcinogen coincident with the decrease in primary carcinoma of the liver?

Finally, a survey of this sort might make an indirect contribution to the problem whether the human liver carcinogen is a chemical conversion product. If carcinogenic activity is found in only part of the liver extracts of a series, all prepared and tested in exactly the same way, this would indicate that if it is a conversion product it is probably not formed from any of the common constituents of liver.

This study was made in an attempt to answer some of these problems by individual extraction and individual testing of liver extracts. Some practical difficulties stand in the way of achieving the answers fully. They include, among others, the following: crudeness of the extracts, so that carcinogenic potency may be hidden; toxicity of some extracts, so that they cannot be tested; causticity of some extracts, so that they slough out and are not given a fair test; small amount of extract obtained from some livers, so that only a few mice can be used in tests.

The animal experiments reported here were begun in 4 groups between July 10, 1940, and May 1, 1941. Consequently the extracts have been under test for periods varying from 20 to 30 months at the time of writing. Although animals are still alive in some of the experiments their number is generally small, as can be seen in the tables. While additional tumors may yet develop their number would probably be small, the optimum time for their induction having passed; thus they could hardly alter the conclusions.

METHODS

Extracts prepared from human livers were tested for carcinogenic activity by injection into mice. In general the procedures were like those previously reported (14, 15) except that each liver was treated as a separate experiment.

Selection of the Cases

Livers from 67 persons were used. Of these 37 were from patients who had a malignant tumor and 30 from noncancerous persons. The 37 cancer cases represent most of the common and some of the rare tumors. No intentional selection was exerted other than that only fresh livers from adults were used, and that, with two exceptions, those used contained few or no metastases. Twenty-seven of the 37 livers had no metastases whatever visible upon either gross or microscopic examination. Eight showed occasional small metastases, generally of microscopic size. One liver weighed 8,319 gm., the increase being due to metastases from a malignant melanoma primary in an eye. It was estimated that this liver was 90 per cent tumor. Another containing a primary carcinoma weighed 3,805 gm., of which it was estimated that 75 per cent was tumor. This avoidance of livers containing tumor metastases was carried out at a time (early in 1940) when it was not yet certain that tumor tissue itself had no carcinogenic factor when tested by this method, as was subsequently reported (15).

The 30 noncancer cases represent a wide variety of common diseases. In addition, 8 of them showed cirrhosis of the liver, and 13 were from Negroes. At least 4 persons among the 30 met violent sudden death at a time when they were apparently in good health; their livers may be considered as near normal as can be obtained from man.

Preparation of the Extracts

The livers were minced by grinding and preserved by freezing or by the addition of one volume of ethanol, as soon as possible after autopsy. They were then saponified and extracted according to the method previously described (14). This consisted essentially of saponification with alcoholic potassium hydroxide on the steam bath for about 24 hours, repeated extraction with ethylene dichloride, and evaporation in partial vacuum. The extract was resaponified once, after which it was recovered as before.

Most of the extracts made have been described in a previous publication (14). The amount of extract from each case, as well as its relation to the type of tumor or other disease, to the location of the tumor, and to the microscopic fatty changes in the liver are given in that paper. The identification numbers used in that report are the same as in the present paper so that those interested may obtain these chemical data. A few extracts there described were not tested in animals and are not mentioned here. Likewise some ex-

tracts are included in the present report that were not previously described because they had not then been prepared. One extract (4945) has been moved from the noncancer to the cancer group because a small mammary gland carcinoma was discovered.

The extracts were prepared for injection by grinding them in a mortar with a solvent which, in 59 cases, was sesame oil. Tricaprylin was used for 7 extracts. One extract required the use of no solvent because it was a heavy oil. The amount of solvent used was generally not enough to dissolve all the extract at room temperature, so they were heated in an oven to 57° C. prior to injection, cooling being permitted in the syringe before the injections were made. Both solvents have been tested and found not to induce tumors.

In this paper the extracts of livers from cancer-bearing persons are hereafter called cancer extracts, while those from persons who had no tumors are called noncancer extracts.

Testing the Extracts

The carcinogenicity of the extracts was tested by subcutaneous injection into mice. The injections were made ventrally in the axillary region. The total amount of extract administered to each mouse was 500 mgm. in 60 of the cases, and 300 mgm. in the remaining 7 cases. This amount of extract was contained in 1.0 to 1.5 cc. of sesame oil, or in 0.6 cc. of tricaprylin. The total dose was given in 1 injection in 24 experiments, 2 injections in 23 experiments, 3 injections in 16 experiments, and 4 injections in 4 experiments. The dose was sometimes subdivided after test injections revealed the extract to be caustic and therefore poorly retained. When more than one injection was given the interval between each was 4 weeks.

The number of mice used in each experiment was governed by the amount of extract available, an attempt being made to use an average of 15. Actually the number varied between 7 and 21 mice. In all, 896 mice were injected with the 67 extracts, of which 456 were given cancer liver extracts and 440 were treated with noncancer extracts.

Two stocks of mice were employed. For testing 11 extracts C57 brown mice were used. They were raised in this laboratory as the first and second generation offspring of breeding stock obtained from the Roscoe B. Jackson Memorial Laboratory. For testing 55 extracts mice of our own partly inbred albino stock were used. Something about their susceptibility to the induction of sarcomas by carcinogenic hydrocarbons and crude tissue extracts and their incidence of spontaneous tumors has been given in several papers (15, 12, 16). The only sarcomas that they bear spontaneously are lymphosarcomas, so far as is known. For 1 extract mice of both stocks were used.

The mice were about equally divided as to sex, 453 females and 443 males. Males were used in 33 experiments, females in 33 experiments, and mice of both sexes in 1 experiment. The mice were kept virgin, with the exception that C57 brown females in 2 experiments were bred.

The mice were given water and a dry mixed diet prepared in this laboratory. The diet appears to be excellent as judged by reproduction, growth, and longevity. It has been kept constant since 1938, except that for several weeks, in 1941, 1 per cent sulfathiazole was added to it. The diet is prepared as follows:

Whole ground corn	45.0 lbs.
Whole powdered milk	20.0 "
Linseed oil meal	10.0 "
Powdered casein	3.1 "
Powdered alfalfa meal	1.8 "
Dried brewer's yeast	1.2 "
NaCl	0.3 "
$CaCO_2$ precipitated	0.3 "

The powdered milk is heated in the autoclave at 5 pounds' pressure for 30 minutes, ground, and added to the mixture.

RESULTS

The early effects of injection of the extracts were those due to their toxicity or to their causticity. The late effects were tumor induction.

For the present only sarcomas that occurred at the site of injection will be considered induced tumors. Tumors developing at distant sites will be presented together as miscellaneous tumors.

Early Effects

A few of the extracts contained a toxic factor so that the mice passed into shock within an hour. Generally they recovered completely within 4 to 6 hours, but in a few experiments the animals quickly passed into coma and died. In one case every mouse died within 4 hours. In another, 11 died of 16 injected. In all, 91 mice of 896 injected died within the first 72 hours after injection. In 33 experiments there were no acute losses of this kind.

Caustic effects of the extracts, when present, appeared mainly between the 5th and the 20th days. They were characterized by extrusion of part of or all the extract through either an ulcer or a sinus. In 12 experiments no or but little extract escaped, in 31 the loss was moderate, and in 23 it was great to total. Some of the extracts in the latter group may be considered to have been inadequately tested. All the experiments are retained, however, in presenting the

results because there was no accurate way of eliminating only some.

There was no relationship between toxicity and causticity. The acute toxic deaths were nearly equally divided between the cancer and the noncancer liver extracts, as was the degree of their causticity.

Incidence of Induced Sarcoma

Twenty-two sarcomas were induced at the site of injection by 14 of the 67 extracts (Tables I and II). As previously stated, some extracts, although they are included here, were not fully tested for their carcinogenic potency because of loss by extrusion. Since 746 mice survived the induction time of 6 months the yield of tumors was 2.9 per cent. Of the 167 mice living at the end of 6 months in the 14 experiments in which sarcomas later appeared, 13.1 per cent subsequently developed sarcomas. This may be considered to be the percentage yield of the effective total tested. Two extracts each induced 3 sarcomas, 4 each induced 2 sarcomas, and 8 each induced 1 sarcoma. About 21 per cent of the extracts were carcinogenic.

The incidence of tumors was considerably less than that previously reported for extracts from pooled specimens (15). Since other factors were the same the most obvious explanation is that one or more of the livers that were pooled might have contained a large amount of the carcinogen.

Induction Time for Sarcomas

The first tumor appeared in the 6th month; this mouse died with sarcoma in the 7th month. The induction time was therefore 6 months. Most of the sarcomas appeared between the 9th and 15th months after injection. Few tumors occurred after this time although a large number of mice were still alive. These time relationships, shown in Table III, resemble those previously reported.

Incidence of Sarcomas Induced by Cancer and by Noncancer Extracts

The extracts that elicited sarcomas were almost equally divided between the cancer and the noncancer cases, as were the number of tumors induced (Table IV). Thus 8 extracts from the 37 tumor cases induced 12 sarcomas in 374 mice surviving for 6 months (2.7 per cent). These results are unlike those previously reported in which an extract from cancer livers induced twice as many tumors as did that from nontumor cases (15).

Relation of the Liver Carcinogen to the Type and Location of the Tumors

The extracts from 8 cancer livers that proved to be carcinogenic were obtained from persons with the following diagnoses: carcinomas of the esophagus, colon, lung, ovary, branchial remnant, or testis; meningioma; and malignant chromophobe tumor of the pituitary. Other extracts from persons with the same diagnoses as the first 4 of these cases were tested and found to be inactive. Therefore, in at least these 4 varieties of tumor there appears to be no specific relationship between the liver carcinogen and the type of tumor that the patient had. All the tumor diagnoses are given in Table I.

The 8 persons whose extracts were carcinogenic all had tumors in different organs, and most of them were in different organ systems. Thus only 2 active extracts were associated with carcinoma of the alimentary tract (esophagus and colon), while at least 8 others from patients with tumor of this system were inactive.

The endocrine system, however, was represented by 3 active extracts, the primary tumors having been in the ovary, testis, and pituitary. To these 3 may be added another extract, from the noncancer cases; namely, that from a case of adenomatous hyperplasia (benign hypertrophy) of the prostate. While not truly a tumor of the endocrine system, it probably results from an imbalance in this system. Thus 4 of the 14 active extracts were related to disease of the endocrine system. In all, 7 extracts from patients with disease of this system were tested and 4 were active. The major diagnoses in the 3 inactive cases were carcinoma of the ovary in 2, and 1 carcinoma of the adrenal.

All the 8 active cancer liver extracts were from patients whose tumors arose from epithelium or neuroepithelium, although others were tested.

The major diagnoses in the 6 noncancer patients whose extracts were carcinogenic were as follows: lobar pneumonia, chronic nonspecific ulcerative colitis, congenital polycystic kidneys, biliary cirrhosis, postherniotomy pulmonary embolism, and adenomatous hyperplasia of the prostate. Except for the last case, there were no obvious differences in the disease, age, and sex between these and the inactive noncancer cases to explain why some were carcinogenic and others were not. Additional data are given in Table II.

Relation of the Liver Carcinogen to the Age of the Patients

The liver extracts that were carcinogenic were obtained from patients in all age groups, whether or not

TABLE I: TUMORS INDUCED BY CANCER LIVER EXTRACTS

Identifying number	Diagnosis	Mice living at time periods, months													Total sarcomas
		0	3	6	9	12	15	18	20	21	23	24	27	30	
H 40-101	Carcinoma, tongue	10	7	5	3	2	2	2	1	1	1	0			0
CCH 40-458	Carcinoma, tongue	17	9	4	3	2	1	1	0						0
4961	Carcinoma, esophagus. Cirrhosis	8	8	7	3	1	1	0*							1
4968	Carcinoma, esophagus	14	14	14	14	14	14	9	9	9	8	7	2	2	0
5016	Carcinoma, stomach	12	12	12	12	11	11	11	11	11	8	8	4	3	0
5037	Carcinoma, stomach	15	12	12	12	12	12	10	10	10	10	9	4		0
5039	Carcinoma, stomach	15	15	15	15	14	10	5	5	3	2	2	2		0
4703	Carcinomas, multiple, bowel	8	4	3	2	1	0								0
4984	Carcinoma, colon	13	13	13	13	12	11	9	7	5	2	2	2	1	0
4990	Carcinoma, colon	14	13	13	11	3**	3	0							2
H 40-378	Carcinoma, liver. Cirrhosis	14	14	13	10	9	5	2	2	1	1				0
4938	Carcinoma, lung	16	15	15	15	15	14	12	8	7	7	5	1	0	0
4948	Carcinoma, lung	13	11	11	10	9	6**	3	2	1	0				2
H 40-186	Carcinoma, lung	12	12	7	1	1	0								0
H 40-183	Carcinoma, prostate	15	4	4	4	3	1	1	1	0					0
H 40-178	Carcinoma, prostate	13	13	13	12	9	7	4	3	2	2				0
H 40-189	Carcinoma, testis	8	8	6	4*	1	1	0							1
5022	Carcinoma, urinary bladder	14	13	11	8	6	2	1	0						0
4945	Carcinoma, breast	9	8	8	8	8	6	5	3	3	3	1	0		0
4958	Carcinoma, breast	11	11	11	10	8	8	6	5	4	3	3	1	0	0
4966	Carcinoma, breast	8	6	5	5	3	2	2	0						0
4991	Carcinoma, breast	17	16	15	9	7	3	2	1	0					0
4927	Carcinoma, ovary	15	12	12	11	11	11	5	4	3	1	0			0
4992	Carcinoma, ovary	11	10	10	10	7	5	4*	3	2	0				1
CCH 40-145	Carcinoma, ovary	6	6	6	6	6	6	4	4	3	3	3	2	0	0
CCH 40-485	Carcinoma, adrenal	19	12	12	9	5	4	1	0						0
4943	Lymphoblastoma	10	10	10	10	9	7	7	7	6	3	3	2	0	0
H 40-185	Lymphoblastoma	16	14	11	8	7	5	2	1	0					0
4974	Reticulum cell sarcoma	7	7	6	6	3	3	2	2	2	1				0
5012	Sarcoma of spleen	10	10	9	8	8	6	4	2	0					0
5014	Acute leukemia	10	9	9	9	9	9	4	4	4	3	3	1	0	0
H 40-231	Hodgkin's lymphogranuloma	15	15	15	15	14	14	7	6	6	5	4	3		0
5013	Meningioma	12	12	12	10	10	8	7	7	7	6	6	2	0*	1
4946	Carcinoma, pituitary, chromophobe	17	15	15	15	14*	9**	4	3	2	0				3
4980	Carcinoma, branchial	8	6	6	6	1*	0								1
H 40-180	Carcinoma, parotid. Cirrhosis	12	12	12	11	10	8	7	5	3	2				0
H 40-184	Malignant melanoma	12	12	12	11	9	8	5	5	5	4				0
	Totals	456	400	374	329	274	223	148	121	100	75	—	—	—	12

* Dead with induced sarcoma. Each asterisk represents one induced sarcoma.

TABLE II: TUMORS INDUCED BY NONCANCER LIVER EXTRACTS

Identifying number	Diagnosis	White or Negro	Mice living at time periods, months												Total sarcomas
			0	6	9	12	15	18	20	21	23	24	27	30	
4964	Lobar pneumonia	W	13	13	13	12	8*	5	4	2*	1				2
4977	Nonspecific ulcerative colitis	W	10	0											0
4985	Nonspecific ulcerative colitis	W	18	17	17	12	11	8	6	5*	4*				2
4940	Abruptio placentae	W	16	15	11	8	6	2	2	1	0				0
4965	Benign hypertrophy of prostate	W	16	15	14*	13	10*	4*	4	4	1	0			3
4971	Syphilis, aortitis, and malignant hypertension	W	19	18	17	12	10	9	7	7	5				0
4973	Polycystic kidneys	W	9	9	9	7*	7	4	0						1
4975	Pneumococcic meningitis	W	15	14	13	12	9	5	3	3	3				0
4979	Hypertensive heart disease	W	4	4	4	1	1	1	0						0
Cor 18-5-40	Lye poisoning	W	15	15	15	13	12	10	7	7	6	4	0		0
4951	Cirrhosis, advanced portal	W	15	12	11	9	7	2	0						0
4960	Cirrhosis, biliary	W	16	15	14	14	12	9	6	6	5	5	2	0*	1
Cor 20-5-40	Cirrhosis, advanced portal	W	15	12	9	9	7	4	3	3	2	2	2	0	0
Cor 65-5-40	Cirrhosis, slight portal	W	15	9	9	9	6	2	2	2	2	2	1	0	0
CCH 40-546	Cirrhosis, advanced portal	N	10	8	8	8	7	6	6	4	3	3	2	0	0
Cor 49-6-40	Cirrhosis, moderate portal	W	15	10	3	1	1	1	0						0
5063	Cirrhosis, cardiac	W	15	12	4	2	1	1	1	1	1	1	0		0
Cor X	Cirrhosis, portal	W	15	15	15	15	14	14	14	14	10	8	4	0	0
Prov 4-1-41	Pulmonary embolism	N	21	18	16	13	8*	7	6						1
Cor 70-3-41	Gunshot wounds of head	N	17	15	11	10	9	8	4						0
CCH 41-331	Cerebral hemmorhage. Tuberculosis	N	16	12	12	12	12	10	10						0
CCH 41-330	Cerebral hemorrhage. Pneumonia	N	16	7	5	5	4	1	1						0
Cor 15-3-41	Hypertension	N	19	18	14	13	11	8	6						0
Prov 3-24-41	Acute appendicitis and peritonitis	N	16	13	12	12	9	8	6						0
Cor 67-2-41	Gunshot wounds of brain	N	16	14	13	11	11	9	6						0
Cor 66-2-41	Knife wounds in neck	N	16	15	15	14	14	12	10						0
Prov 500	Congestive heart failure	N	11	11	7	6	4	2	1						0
Cor 55-12-40	Ether death	N	10	10	10	9	5	3	1						0
Prov 3-19-41	Pyelonephritis	N	16	15	8	6	5	4	2						0
Cor 83-4-41	Stab wounds in neck	N	15	11	10	9	7	3	3						0
	Totals		440	372	319	277	228	162	122	—	—	—	—	—	10

* Dead with induced sarcoma. Each asterisk represents one induced sarcoma.

they had cancers (Table V). There is clearly no increase in the carcinogenic substance in the liver with increase in age, as indicated either by the number of extracts that were carcinogenic or by the number of tumors that they induced.

TABLE III: TIME OF INDUCTION OF SARCOMAS

Time after injection, months	Living mice	Number dead with induced sarcomas
0	896	0
3	781	0
6	746	0
9	648	2
12	551	5
15	451	7
18	310	3
21	—	2
24	—	1
27	—	0
30	—	2

TABLE IV: INCIDENCE OF SARCOMAS INDUCED BY CANCER AND BY NONCANCER EXTRACTS

Source of the extract	Number of extracts tested	Number of extracts inducing sarcomas	Extracts inducing sarcomas, per cent	Number of mice living at 6 months	Number of sarcomas induced	Mice having sarcomas, per cent
Tumor cases	37	8	21.6	374	12	3.2
Nontumor cases	30	6	20.0	372	10	2.7
Totals	67	14	—	746	22	—

TABLE V: RELATION OF THE LIVER CARCINOGEN TO THE AGE OF THE PATIENTS

Age, years	Cancer cases: Number of extracts	Cancer cases: Number of extracts inducing sarcomas	Cancer cases: Number of sarcomas induced	Noncancer cases: Number of extracts	Noncancer cases: Number of extracts inducing sarcomas	Noncancer cases: Number of sarcomas induced
20–29	1	1	1	4	0	0
30–39	3	1	1	4	1	1
40–49	11	1	3	9	3	4
50–59	9	2	3	6	0	0
60–69	8	1	1	5	2	5
70–79	5	2	3	0	0	0
Unknown	0	0	0	2	0	0
Totals	37	8	12	30	6	10

The average age of the cancer patients was 53.0 years, while that of those noncancer patients in whom the age is known was 46.4 years. Since 8 extracts from the 37 cancer patients and 6 from the 30 noncancer patients were carcinogenic, by this method of analysis, again, carcinogenic activity did not increase with age.

RELATION OF THE LIVER CARCINOGEN TO THE SEX OF THE PATIENTS

The liver extracts that proved to be carcinogenic were obtained in about the same proportion from the 2 sexes, if allowance is made for the preponderance of males. This is true for extracts from cancer patients, and probably also from noncancer patients (Table VI).

RELATION OF THE LIVER CARCINOGEN TO RACE

Extracts were prepared from 12 Negroes without cancer, from 1 with cirrhosis, and from 2 who had cancer, a total of 15. In selecting these cases only very dark Negroes were used in order to avoid, as far as possible, those of mixed race. Only 1 of these extracts was carcinogenic (Table VII). In comparison, 13 extracts of 52 from white persons contained the carcinogenic factor. While these differences appear significant it is possible that they are not, for two reasons: First, for some unknown reason many extracts from Negroes were poorly retained, half of them having shown great loss. The 4 livers that were most nearly normal, taken from healthy Negroes who died sudden, violent deaths from gunshot and knife wounds, and from 2 others who died suddenly, all yielded extracts that showed the maximum degree of causticity with ulceration. This observation remains without explanation. Second, 12 of these Negro liver extracts have been under test for only 20 months and numerous mice are still living. If one additional extract were to induce a tumor in the future, these extracts would have their full quota.

TABLE VI: RELATION OF THE LIVER CARCINOGEN TO THE SEX OF THE PATIENTS

Sex	Cancer cases: Number of extracts	Cancer cases: Number of extracts inducing sarcomas	Cancer cases: Number of sarcomas induced	Noncancer cases: Number of extracts	Noncancer cases: Number of extracts inducing sarcomas	Noncancer cases: Number of sarcomas induced
Male	24	4	7	20	5	8
Female	13	4	5	9	1	2
Unknown	0	0	0	1	0	0
Totals	37	8	12	30	6	10

TABLE VII: RELATION OF THE LIVER CARCINOGEN TO RACE

Race	Number of extracts	Number of extracts inducing sarcomas	Number of mice alive at 6 months	Number of sarcomas induced
White	52	13	560	21
Negro	15	1	186	1
Totals	67	14	746	22

The Negro whose liver extract was carcinogenic was a male, aged 37, who died of pulmonary embolism after a herniotomy. His liver showed neither cirrhosis nor hepatitis. In this test 18 mice survived for 6 months, and 6 are still alive but without tumor at the time of writing (20 months).

The liver of the American Negro, then, is unlike that of the African Bantu in that it does not contain detectable carcinogenic activity in a greater proportion of cases than does that of white persons. It may actually contain less.

Relation of the Liver Carcinogen to Cirrhosis

Extracts were tested from 8 persons who had a significant degree of cirrhosis of the liver, and from 3 with cirrhosis in addition to a fatal cancer, a total of 11. Several livers that showed a minimal amount of cirrhosis, visible only on microscopic examination, are classified with the noncirrhotic livers. The cirrhoses were mostly portal, but one biliary and one cardiac cirrhosis are included. The cirrhosis was the major factor in most of the deaths. One cirrhotic liver contained a primary carcinoma.

Table VIII: Relation of the Liver Carcinogen to Cirrhosis

	Number of extracts	Number of extracts inducing sarcomas	Number of mice surviving for 6 months	Number of sarcomas induced
Cirrhotic	11	2	125	2
Noncirrhotic	56	12	621	20
Totals	67	14	746	22

Two of these extracts were carcinogenic (Table VIII). One was from a white male, aged 42, with biliary cirrhosis, the other from a white male, aged 35, who had a mild degree of portal cirrhosis and who died of a carcinoma of the esophagus. These 2 extracts induced 1 sarcoma each, 15 mice surviving for 6 months in the first experiment and 7 in the latter.

Cirrhotic liver, then, did not show an abnormal amount of the carcinogenic factor.

Relation of Carcinogenicity to the Total Amount of the Extract

The average amount of extract obtained from the 14 livers that showed carcinogenic activity was 9.7 gm. In contrast, the average amount from noncarcinogenic extracts, in which the data are available, was 13.3 gm. This difference appears to be important, but its interpretation is uncertain. It might signify merely that voluminous extracts diluted their carcinogen so that it was not detected on testing. Against such an interpretation stands the fact that 3 of the 13 extracts that were carcinogenic weighed more than the average noncarcinogenic extracts.

Relation of the Number of Injections to the Number of Induced Sarcomas

Although the total amount of extract injected was the same in all experiments, namely 500 mgm. per mouse, except in 7 experiments in which it was 300 mgm., it was sometimes administered in multiple injections, given at intervals of 4 weeks. The caustic extracts had to be so subdivided to obtain retention. The influence of this procedure on tumor induction is presented in Table IX. It appears that increasing the number of injections had slight, if any, effect on the number of cases showing tumors, but that with a greater number of injections the number of tumors induced may actually have decreased.

With one exception, the 14 extracts that induced tumors showed less than the average loss from caustic action on the skin. Also, in the same 14 experiments the mice generally showed large amounts of the extract persisting at autopsy. In many of the experiments in which the extracts failed to induce sarcomas little or no residue was seen at autopsy. Subdivision of the extract for injection was only partially effective in preventing its loss. This appears to explain the failure to induce more tumors with an increase in the number of injections.

Table IX: Relation of the Number of Injections to the Number of Induced Sarcomas

Number of injections	Number of experiments	Number of experiments showing sarcomas	Total sarcomas
1	24	6	12
2	23	7	9
3	16	1	1
4	4	0	0
Totals	67	14	22

The first injections appeared to be neither more nor less caustic than were subsequent injections. There was no evidence for the development of anything in the nature of an Arthus or sensitizing reaction.

The number of injections, then, did not appear to influence the number of sarcomas induced, but it did signify roughly the degree of causticity of the extracts.

Relation of the Stock of Mice to the Number of Induced Sarcomas

Both stocks of mice gave rise to sarcomas. The C57 browns, used in 11 experiments in which 133 mice were injected, of which 115 survived for 6 months, gave rise to 2 sarcomas in 2 experiments. The albino stock, used in testing 55 extracts, 763 mice having been injected, of which 631 lived for 6 months, yielded

20 sarcomas in 12 experiments. One extract, injected into mice of both stocks, induced no tumors. The C57 browns appear to have been slightly less susceptible to the induction of tumors, but the figures are probably not significant. The skin of the C57 browns is thinner than that of the albino stock, and they retained less of the extract, as was revealed at necropsy.

Relation of the Sex of the Mice to the Number of Induced Sarcomas

Tumors were induced in mice of both sexes (Table X). In all 443 male mice were used. They gave rise to 13 sarcomas, while 453 female mice yielded 9 sarcomas. Males were used in 33 experiments, in which 7 developed sarcomas, and females in 33 experiments, with sarcomas occurring in 7. Mice of both sexes were used in 1 experiment. Males gave rise to more sarcomas than did females, a result in conformity with that previously reported (15). Both the sarcomas induced in C57 brown mice were in females.

Table X: Relation of the Sex of the Mice to the Number of Induced Sarcomas

Sex of mice	Number of mice injected	Number of sarcomas induced	Number of extracts injected	Number of extracts inducing sarcomas
Male	443	13	33.5	7
Female	453	9	33.5	7
Totals	896	22	67	14

Relation of the Solvent to the Number of Induced Sarcomas

All the sarcomas induced occurred in experiments in which sesame oil was used as the solvent. No sarcomas were elicited in the 7 experiments in which tricaprylin was used. It is hard to interpret this result because of the small number of cases in which tricaprylin was used, and because in the same 7 experiments the amount of extract administered was reduced to 300 mgm. The possibility, however, that sesame oil had a cocarcinogenic action cannot be excluded.

Morphology of the Induced Sarcomas

The sarcomas induced by these extracts resembled those induced by carcinogens of known chemical composition, and by other tissue extracts. They grew rapidly after they appeared, killing the host in from 4 to 6 weeks. They were firm or hard, smooth in contour, and early became fixed to the underlying muscles and soon thereafter also to the overlying skin, through which some of them ulcerated. Part of or all the extract remaining was often enclosed by the tumor.

Microscopically they were spindle or mixed cell sarcomas with no unusual features.

Miscellaneous Tumors

In the course of these experiments some mice developed tumors of the lungs, lymphatic system, mammary glands, and occasionally elsewhere. The problem arises whether they should be called induced tumors. A safe rule seems to be that if the incidence of tumors in sites distant from the point of injection of the substance being tested is significantly greater than in suitable controls, the excess may be counted as induced.

Table XI: Comparison of the Number of Miscellaneous Tumors in the Cancer and Noncancer Liver Extract Experiments

Type of liver extract	Number of extracts	Number of mice injected	Number of mice alive at 12 months	Number of mice alive at 18 months	Lung tumors	Lymphatic tumors	Mammary tumors
Cancer livers	37	456	274	148	40	20	32
Noncancer livers	30	440	277	162	36	18	34

Table XII: Comparison of the Number of Miscellaneous Tumors in Experiments with Sarcoma-Inducing and Non-Sarcoma-Inducing Liver Extracts

Types of liver extract	Number of extracts	Number of mice injected	Number of mice alive at 12 months	Number of mice alive at 18 months	Lung tumors	Lymphatic tumors	Mammary tumors
Sarcoma-inducing extracts	14	184	117	55	20	10	10
Non-sarcoma-inducing extracts	53	712	434	255	56	28	56

In Table XI the number of miscellaneous tumors (lung, lymphatic system, and mammary gland) in the mice injected with the cancer extracts is compared with those in the mice injected with the noncancer liver extracts. There was no difference in the incidence of tumors at distant sites in these 2 groups.

In Table XII the miscellaneous tumors are analyzed in another way. Here the 67 liver extracts are divided into 2 groups; namely, those that were carcinogenic at the point of injection and those that were not. The number of miscellaneous tumors in the 2 groups is then compared. It is seen that the mammary tumors were surely not increased by the carcinogenic liver extracts, and the lung and lymphatic tumors probably not.

No data are available on the incidence of miscellaneous tumors in a large group of the albino mice subjected to no experimental procedure. The best controls available are mice used for testing various materials that proved to be not locally carcinogenic (Table XII). Additional similar control data may be found in previous publications. Thus in a total of 229 mice injected with extracts of human tissues, 159 mice survived for 12 months, 79 for 18 months, and 24 for 24 months. The miscellaneous tumors in these mice were: 59 lung, 41 lymphatic, and 26 mammary gland. These data are taken from reference 15, experiments 5 to 9, Tables II and IV. Similar data taken from another publication (see reference 16, experiments 1 to 6 and 13 to 16, Tables I and II) and giving the outcome in mice injected or fed with extracts of animal tissues show essentially the same incidence of miscellaneous tumors. Of 201 mice injected, 120 lived for 12 months, 64 for 18 months, and 17 for 24 months. They developed 43 lung, 22 lymphatic, and 23 mammary tumors.

The miscellaneous tumors in the C57 brown mice consisted of 5 in the lymphatic system and 4 in the mammary gland. The latter were all found in one group of females that were bred. In all, 133 C57 brown mice were injected (99 females and 34 males). They showed the following survival time: 115 were alive at 6 months, 86 at 12 months, 58 at 18 months, and 50 at 21 months. Certainly the extracts were not highly carcinogenic at distant sites in these C57 brown mice.

It may be safely concluded from these combined data that the incidence of miscellaneous tumors was approximately the same in mice injected with liver extracts that were locally carcinogenic and with those that were not. Furthermore, the incidence of the tumors at distant sites was about the same in the present experiments as in others in which the same stock of mice was injected with locally noncarcinogenic human and animal tissue extracts. It cannot be stated that there was no increase whatever in the incidence of miscellaneous tumors in any of these experiments.

COMMENTS

This survey has given much new information about the carcinogenic activity found in human liver. At the same time it has produced some results confirming those previously reported by the writers and by others, some that supplement previous work, and some that contradict older results. An attempt will now be made to explain and harmonize these differences.

The presence of a carcinogenic factor in extracts of human liver has again been confirmed. The induction time remains about the same, namely 6 months. The factor was present in only about 21 per cent of the extracts tested. The percentage yield reported here (2.9 per cent) is much less than that given in an earlier experiment (32.4 per cent), but it compares favorably with the incidence reported by other workers (7, 4, 6, 10, 8). The reasons for the present lower percentage yield are unknown, although several explanations present themselves: (a) It is possible that one or more of the livers that were pooled for extraction was very rich in the carcinogenic substance and contributed it to the whole extract. (b) The extracts reported here were injected ventrally, whereas those reported previously were given dorsally where the skin is tougher. It is possible that enough more of the extract was lost to account for the lower tumor incidence. In these experiments about one-third of the extracts produced severe ulceration, and many were inadequately tested in consequence.

In this survey the carcinogenic activity was found both in livers from persons who had cancer and those who had not. This confirms the earlier statements by the present author (15) and by des Ligneris (4). The results are unlike those of other groups of workers, who induced no tumors at the site of injection with noncancer liver extracts, but who showed that carcinogenic activity was possibly present because the incidence of various types of tumors at distant sites was greater than in controls (7, 10, 8).

In the present series of experiments the carcinogen in the liver appeared to be particularly prevalent in those whose tumors were related to the endocrine system. Aside from this observation, there seems to have been no special relation to any type of major disease, whether tumor or nontumor. The carcinogen was not present in 3 liver extracts from patients with carcinoma of the stomach, contrary to the observation of Schabad (11). Neither was it present in 4 extracts from persons with malignant lymphatic diseases of several types, unlike the observations of Hieger (6). It seems that many more tests must be made before the presence of the carcinogen can be correlated accurately with the major disease.

The carcinogen was not more common in the livers of American Negroes than in white persons, which is unlike the results reported by des Ligneris for the Bantu. This observation, if it is correct, can be interpreted in several ways. It might mean that a liver carcinogen, when present, accounts for the high incidence of primary hepatic carcinoma reported for the Bantu, and that, when absent, for the low incidence of this tumor in the American Negro and others. Also, it may mean that the liver carcinogen in the Bantu is there because of environmental factors, and not because of any inherent racial disorder of metabolism.

The carcinogen was not commoner in cirrhotic than in noncirrhotic livers. This seems to indicate that it is not the cause of the cirrhosis. It may or may not account for the origin of cancer in cirrhosis since primary carcinoma of the liver often begins in cirrhotic livers, but many cirrhotic livers do not become cancerous. The one liver that had both cirrhosis and a primary carcinoma was inactive.

These experiments make no contribution to the question whether the carcinogenic activity in the liver extracts is due to the presence of a preformed carcinogen or to a substance made active by the chemical procedures. The point cannot be settled until the carcinogen is identified, a problem toward which efforts are now directed, unless methods for extraction and concentration are found that are free of all criticism. Schabad used simple benzene extraction, but the extracts so obtained were only mildly carcinogenic, possibly because of the large amount of extraneous material that they contained. The carcinogen might be of great importance even if it does not exist in the body in a preformed state. If carcinogenic activity can be conferred on substances in the liver by the simple procedures used, this observation alone might be important. The fact that only 21 per cent of a series of livers, all tested in the same way, contained carcinogenic activity speaks against its being formed from a normal constituent of liver.

Despite its deficiencies this survey yielded much information, which will be helpful in guiding further work on the carcinogenic factor in human liver. It is to be emphasized again that the results and interpretations are tentative, that another survey will be necessary when the technical methods are improved, and that some of the results might be changed when more cases have been studied. For these reasons only a summary is offered, and no conclusions are drawn.

SUMMARY

Individual extractions and tests for carcinogenicity performed on 67 human livers, in which 896 mice were used, gave the following results:

1. The presence of a carcinogenic factor in extracts of human liver was again demonstrated. It was present in livers from both cancer and noncancer persons.

2. The yield of induced sarcomas was 2.9 per cent in all mice that survived for 6 months. It was 13.1 per cent in those experiments in which sarcomas were induced. Fourteen extracts out of 67 tested (about 21 per cent) had carcinogenic activity.

3. The induction time for the first tumor was 6 months.

4. Eight extracts from cancer patients of 37 tested were carcinogenic (21.6 per cent), while 6 of 30 noncancer extracts were carcinogenic (20.0 per cent).

5. Carcinogenic activity in the extracts was not related to any special site of tumor, or to any type, except that 4 of the 14 active extracts were from persons whose major disease was related to the endocrine glands concerned with steroid hormones.

6. The incidence of the carcinogenic factor was about equal in all age groups, in the two sexes, in whites and Negroes, and in cirrhotic and noncirrhotic livers.

7. The livers that showed the carcinogenic factor contained less extract on the average than did those that were inactive.

8. Other experimental factors, including the number of injections, the stock of mice, and possibly the type of solvent, did not appear to influence the results.

9. The sarcomas were spindle and mixed cell, with no unusual features.

10. The number of tumors at distant sites was not increased over those in several control groups.

11. The carcinogenic activity in extracts of human liver has not been shown to be due to a preformed carcinogen, although this possibility is not excluded. It might be of importance even if it were a chemical conversion product.

REFERENCES

1. Berman, C. The Etiology of Primary Carcinoma of the Liver with Special Reference to the Bantu Races of South Africa. South African J. M. Sc., **6**:145-156. 1941.
2. Berman, C. Primary Carcinoma of the Liver in the Bantu Races of South Africa. An Analysis of the Incidence of Primary Carcinoma of the Liver amongst the Bantu on the Witwatersrand Gold Mines and in Johannesburg with Special Reference to the Incidence of This Disease amongst Other Races. South African J. M. Sc., **5**:54-72. 1940.
3. Bonne, C. Cancer and Human Races. Am. J. Cancer, **30**: 435-454. 1937.
4. des Ligneris, M. A. J. The Production of Benign and Malignant Skin Tumours in Mice Painted with Bantu Liver Extracts. Am. J. Cancer, **39**:489-495. 1940.
5. Gummel, H. Beitrag zur Frage des Nachweises körpereigener cancerogener Substanzen. Klin. Wchnschr., **20**: 448-449. 1941.
6. Hieger, I. The Examination of Human Tissue for Carcinogenic Factors. Am. J. Cancer, **39**:496-503. 1940.
7. Kleinenberg, H. E., Neufach, S. A., and Schabad, L. M. Endogenic Blastogenic Substances. Am. J. Cancer, **39**: 463-488. 1940.
8. Kleinenberg, H. E., Neufach, S. A., and Schabad, L. M. Further Study of Blastomogenic Substances in the Human Body. Cancer Research, **1**:853-859. 1941.
9. Lewis, J. H. The Biology of the Negro. Pp. 433. Chicago: The University of Chicago Press. 1942.
10. Sannié, Ch., Truhaut, R., and Guérin, P. Production de sarcomes chez la souris par injections d'un extrait obtenu à partir de foies de malades cancéreux. Bull. Assoc. franç. p. l'étude du cancer, **29**:106-121. 1941.

11. Schabad, L. M. Production expérimentale de tumeurs malignes par un extrait benzénique du foie d'un cancéreux. A propos de la question des substances cancérigènes endogènes. Compt. rend. Soc. de biol., **124**: 213-216. 1937.
12. Steele, R., Koch, F. C., and Steiner, P. E. The Extraction of a Carcinogenic Fraction from Human Urine. (Preliminary Report.) Cancer Research, **1**:614-617. 1941.
13. Steiner, P. E. A Cancerogenic Tissue Extract from Human Sources. Science, **92**:431-432. 1940.
14. Steiner, P. E. The Nonsaponifiable Lipid Fraction of Livers from Cancerous and Noncancerous Persons. Am. J. Path., **17**:667-671. 1941.
15. Steiner, P. E. The Induction of Tumors with Extracts from Human Livers and Human Cancers. Cancer Research, **2**:425-435. 1942.
16. Steiner, P. E., Steele, R., and Koch, F. C. The Possible Carcinogenicity of Overcooked Meats, Heated Cholesterol, Acrolein, and Heated Sesame Oil. Cancer Research, **3**: 100-107. 1943.

The Effect of Rate of Freezing on the Survival of Fourteen Transplantable Tumors of Mice*

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Since the discovery that many transplantable tumors will survive freezing and thawing, considerable attention has been devoted to the effect of the rate of the two processes on the percentage of survival. The results of different investigators have not agreed very well, probably in large part because of the variety of tumors that have been used and differences in the methods by which "rapid" and "slow" freezing and thawing have been accomplished. Recent papers (1, 6) include excellent reviews of the literature, so that only a few of the more important articles need to be mentioned here.

Breedis, Barnes, and Furth (2) reported that slow freezing favored survival. Slow freezing was accomplished by sealing the tumor tissue (leukemic cells suspended in Tyrode solution) in a test tube, immersing this in ether at 0° C., and cooling to a temperature of approximately −70° C. over a period of 10 to 20 minutes by the addition to the ether of small pieces of solid carbon dioxide (dry ice). For fast freezing, the tube containing the leukemic cells was plunged directly into chilled ether. With this method, the contents of the tube could be brought to −69° C. or lower in 60 seconds.

In a later paper, Breedis and Furth (3) reported the successful preservation of "all neoplastic cells so far tested" when the cooling process was slow (1 hour for the drop from 0° C. to approximately the temperature of dry ice) and the thawing rapid.

A further analysis (1) indicated that, at least in the case of leukemic cells, the rate of cooling must in fact be very rapid to be damaging, and that a moderate retardation of the process is just as effective in preserving the life of the cells as is a much greater retardation. The most rapid freezing was accomplished by dropping the cell suspension, one drop at a time, on the inner wall of a test tube immersed in liquid nitrogen. This completely inactivated all three tumors used. A variety of methods of slower freezing was also used. In one experiment immersion of cell suspensions in a test tube (already chilled to 0° C.), in ether at −15°, −25°, or −35° C. was without deleterious effect; however, if the temperature was −45° C. or lower some damage resulted. It was concluded that the temperature of suspensions at their centers must drop from 0° C. to −15° C., the range in which actual freezing occurs, in 12 seconds or less for significant damage to result as compared with the damage done by freezing at any slower rate. Freezing in 1 minute and freezing in 30 minutes were found to be equivalent.

Mider and Morton (6) worked with two mouse sarcomas and a rat carcinoma. As a method of fast freezing they immersed cell suspensions or solid masses of cells, sealed in a test tube, in a mixture of methyl cellosolve and solid CO_2 at about −74° C. This lowered the temperature of the tissue to −74° C. in 3 to 5 minutes. The time required just to freeze the tissue was not determined. For slow freezing, cells similarly sealed were cooled to −74° C. over a period of 20 minutes by the gradual addition of dry ice to the methyl cellosolve.

When cell suspensions were employed, fast freezing was apparently more deleterious than slow. However, the number of animals used was too small to permit definite conclusions. When cell masses were used, no difference between the effects of fast and slow freezing was noted, except that if the freezings were repeated several times the multiple slow freezings were the more lethal.

Mider and Morton's data bring out one additional interesting point; namely, that, at least in the case of the tumors used, cells frozen *en masse* survive better than cell suspensions.

Perhaps the most clear cut evidence as to the effect of the rate of freezing has been provided by an entirely different method of observation; namely, the Altmann technic of freezing-drying (7). Tissues were frozen by various methods, then run through a freezing-drying process, sectioned, and stained. The clearest results were obtained from tissues frozen by immersion in isopentane chilled in liquid nitrogen to temperatures ranging from −60° to −195° C. At −195° C., three zones were clearly differentiated: (a) On the surface, and hence in the region of fastest freezing, a narrow zone of superior preservation without evidence of damage by ice crystals. (b) A some-

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what broader zone just below the surface in which maximum damage was evident. The cells showed serious artifacts, presumably owing to the action of ice crystals. (c) An innermost zone of slowest freezing, with somewhat better preservation than zone b, though still showing evidence of damage by ice crystals. Variation between cells in this zone was considerable. In a series of tissues frozen at various temperatures above −195° C. the zones were pushed toward the surface by an amount corresponding to the elevation of the temperature. At −60° C. the whole tissue showed the characteristics of zone c. At −100° to −140° C. zone a was absent, zone b at the surface.

METHODS AND MATERIALS

Guided by the report (3) that cooling of tumor tissue for 1 hour was advantageous to survival of the frozen tissue, and by the observation (4) that spermatozoa are subject to a "shock effect," being damaged by too rapid cooling in the zone from 37° C. to 0° C., we carried out experiments to see if the damaging effect of rapid cooling was confined to some one temperature zone. Rapid cooling was accomplished by dropping a 1 cc. vial containing a small piece of tissue directly into water and ice at 0° C., or into salt water and ice at −5 to −10° C., or directly onto solid CO_2. All combinations of rapid cooling, induced as above over certain intervals, combined with slow cooling over the remaining intervals, gave essentially the same percentage of survival with a considerable variety of tumors.[1] Consequently attention was turned to more rapid methods of freezing. Three were finally adopted, as follows:

1. "Ordinary" freezing.—A small piece of tumor was placed in a 1 cc. vial and the vial was stoppered and dropped directly on dry ice in a 1 gallon thermos jug. With this method pieces of tissue 3 or 4 mm. in diameter were frozen in about 1 minute. This was measured by removing vials from the dry ice after various intervals, quickly breaking them, and cutting through the tissue with a razor blade. If the tissue was white and hardened, it was regarded as frozen.

2. Slow freezing.—The tissue was cut with scissors into thin slices (averaging about 1×2×6 mm.). A vial ⅞ inch in diameter was filled to a depth of 1 to 1¼ inches with freon 11 (CCl_3F)[2] or isopentane at room temperature. The tissue was dropped into the fluid, and the corked vial with its contained fluid and tissue placed for about 5 minutes on the surface of dry ice in a gallon thermos jug, then buried to the level of the cork in the dry ice. Freezing by this method was probably slightly slower than by the ordinary method, a temperature of −10° C. being reached in about 3 minutes. In 30 minutes the temperature reached −76° C.

After the first few tests, only isopentane was used because its slightly higher boiling point made it more convenient to handle.

3. Fast freezing.—Tissue prepared as for method 2 was dropped directly into isopentane at approximately the temperature of dry ice. Freezing was complete in from 1 to 2 seconds, as determined by holding tissue for a timed interval in the chilled isopentane with forceps, quickly removing, and cutting through with a razor blade.

In two experiments we also froze tissues by the method of Breedis and Furth (3), repeated exactly except that a slightly longer test tube was used and that Locke's was substituted for Tyrode's solution.

In all experiments here reported, tissues after freezing were kept on dry ice for ½ to 1 hour, then thawed by immersion in Locke's solution at 33–38° C., and promptly implanted with a trocar under the skin near the right foreleg.

Animals were not discarded as negative until at least 8 weeks after inoculation.

The tumors used will be described in an article now in preparation.

RESULTS

Since the method of freezing adopted involved immersion of the tumor tissue in freon 11 or isopentane, it was necessary to run tests to determine the deleterious effects, if any, of this treatment. The results are shown in Tables I and II. The figures in Table I show that, at least for the limited number of tumors tested, immersion up to 30 minutes at room temperature caused little if any damage, immersion for 1 hour was definitely damaging, immersion for 24 hours lethal. Table II shows that there was no consistent difference in survival following slow freezing in isopentane as compared with "ordinary freezing." There is a suggestion that leukemias were more subject to damage by the isopentane than were other tumors, but more data would be necessary to confirm this.

It should be emphasized that the tests summarized in Table I were carried out at room temperature. There is every reason to suppose that at lower temperatures less damage would result from the same length of exposure. The slow freezing method, furthermore, might be expected to permit more rapid and more harmful physical and chemical action by the isopentane than the fast method.

It having been established by these preliminary tests that 30 minutes' immersion in isopentane or freon 11 was not significantly detrimental for the 3 tumors tested, 14 tumors of a wide variety of types were selected and employed to study the relative dam-

[1] We are greatly indebted to Mr. Joshua Burnett for aid in this series of experiments.

[2] We are indebted to Kinetic Chemicals, Inc., of Wilmington, Delaware, for providing the freon 11.

TABLE I: SURVIVAL OF TUMORS EXPOSED TO FREON 11 AND ISOPENTANE AT ROOM TEMPERATURE

Tumor	Tumor type	Exposed to	Duration, minutes	Number of animals	Takes, per cent
dbrB	Mammary carcinoma	Freon 11	30	5	100
15091a	Mammary carcinoma	Freon 11	30	5	100
15091a		Isopentane	30	5	100
LL493	Monocytic leukemia	Freon 11	1 hours	4	100
LL493		Freon 11	1	4	50
LL493		Freon 11	24	5	0

TABLE II: COMPARISON OF ORDINARY FREEZING AND SLOW FREEZING IN ISOPENTANE

Tumor	Tumor type	Tumor generation	Takes	
			Ordinary, per cent	Isopentane, per cent
E0771	Mammary carcinoma	11	78	
		12, 13, 20, 22		93
S91	Melanoma	23	63	
		32		100
P1534	Lymphoid leukemia	5?	86	
		17		40
P1679	Lymphoid leukemia	7		0
		8	100	
		9	67	17
C1498	Myeloid leukemia	15	0	0
P1643	Myeloid leukemia	6	0	0

TABLE III: GROWTH OF TRANSPLANTED TUMORS FOLLOWING FAST AND SLOW FREEZING IN ISOPENTANE

Tumor	Tumor type	Tissue used for inoculation	Tumor generation	Slow freezing			Fast freezing		
				Number of animals	Takes, per cent	Average lag	Number of animals	Takes, per cent	Average lag
P1743	Lymphoid leukemia	Spleen	4	6	0		6	0	
P1643	Myeloid leukemia	Subcut. mass	5	5	0		6	0	
P1679	Lymphoid leukemia	Subcut. mass	7	5	0		6	0	
P1679			9	6	17	22.0	6	0	
C1498	Myeloid leukemia	Spleen	15	6	0		6	0	
P1534	Lymphoid leukemia	Subcut. mass	17	5	40	24.0	6	0	
P1019	Teratoma	Subcut. mass.	26	5	0		6	0	
S91	Melanoma	Subcut. mass	32	5	100	25.8	5	80	40.0
C198 *	Reticuloendothelioma	Liver	72	5	100	29.6	5	20	27.0
C198			82	22	54.5	28.4 ± 1.5	25	4	27.0
E0771 *	Mammary carcinoma	Subcut. mass	12	6	100	19.0	6	67	13.0
E0771			13	6	50	26.3	6	17	41.0
E0771			20	25	100	20.2 ± 1.3	23	100	15.1 ± 0.8
E0771			22	21	95	21.9 ± 1.4	23	100	13.3 ± 1.0
E060	Mammary carcinoma	Subcut. mass	140	5	100	8.2	5	80	14.0
15091a	Mammary carcinoma	Subcut. mass	311	5	100	9.2	5	100	10.2
15091a †			311	5	100	9.0	5	100	10.8
dbrB	Mammary carcinoma	Subcut. mass	> 500	5	100	10.8	5	100	19.6
dbrB †			> 500	5	100	?	5	80	?
L946AII *	Fibrosarcoma, bone	Subcut. mass	66	6	67	5.7	6	100	8.7
L946AII *	origin		67	6	100	12.0	6	100	7.5
L946AII			68	6	100	10.2	6	100	11.0
C252	Fibrosarcoma	Subcut. mass	124	5	100	5.8	5	40	20.5
C252			134	25	100	5.7 ± 0.2	25	96	12.4 ± 1.5

* Tissue cut very thin.

† Frozen in freon 11 instead of isopentane.

age produced by fast as compared with slow freezing in isopentane. Two methods of measuring damage were employed; namely, (a) the percentage of takes, (b) the average lag. Lag was defined as the interval in days between inoculation and the first appearance of an unmistakable palpable mass, usually about 2 mm. in diameter. Observations were made daily for the first few days when growth might be expected, then every other day, usually for a week or more, then at longer intervals.

The results are summarized in Table III. They indicate that for most tumors, fast freezing (in 1 to 2 seconds) was more detrimental than slow (in about 10 minutes). Of 13 cases in which there was a difference, 11 showed a lower percentage of takes following fast freezing, 2 following slow freezing. As for average lags, of 16 cases 10 showed a greater lag following fast freezing, 6 following slow freezing. In most instances the number of animals was too small for the individual cases taken alone to be significant. In 4 cases involving 3 tumors, 44 to 50 animals, divided between the fast and slowly frozen groups, were used. These cases are naturally the most instructive.

Tumor C198 grew in 12 of 22 animals inoculated following slow freezing, 1 of 25 animals inoculated following fast freezing. An earlier experiment with fewer animals showed a similar trend. Owing to the small number of takes with fast freezing the difference in the lags is not significant. The detrimental effect of fast freezing is unquestionable.

Tumor C252 grew in 25 of 25 animals inoculated following slow freezing, 24 of 25 animals inoculated following fast freezing. The average lags were 5.7 ± 0.2 and 12.4 ± 1.5 respectively. The difference is significant and shows fast freezing to have been the more detrimental. An earlier test with the same tumor gave similar results.

Tumor E0771 was tested 4 times, in the 12th, 13th, 20th, and 22nd tumor generations. Of 91 animals used in the last two tests, in only 1, a mouse inoculated with slowly frozen tissue, did the tumor fail to grow. This is contrary to the usual situation, but not statistically significant evidence of greater damage by slow freezing. However the average lags in the two experiments were 20.2 ± 1.3 and 21.9 ± 1.4 respectively with slow freezing, 15.1 ± 0.8 and 13.3 ± 1.0 respectively with fast freezing. Although the difference is less definite than with C252, it is significant and in the reverse direction, slow freezing being more detrimental. Curiously enough in the two tests carried out in the 12th and 13th tumor generations, fast freezing gave fewer takes than slow freezing. Although the numbers were too small in these two tests to permit definite conclusions, it is not improbable that the difference is real, and due to the occurrence of a mutation or mutations between the 13th and 20th tumor generations.

Results with other tumors need not be discussed in detail. Suffice it to say that an examination of the data in Table III will show that tumors P1679, P1534, S91, E060, dbrB, and perhaps 15091a were probably more damaged by fast than by slow freezing, while of L946AII the reverse is perhaps true.

Two experiments were run to compare the survival of tumors frozen by Breedis and Furth's recommended slow method (3) and by our "ordinary" method. Though not sufficiently extensive for a generalization the data, summarized in Table IV, show no advantage of one over the other. We have used our very simple ordinary method with success for a number of tumors, keeping some of them for long periods. Some tumors apparently will not survive freezing by any method.

TABLE IV: COMPARISON OF BREEDIS AND FURTH METHOD WITH "ORDINARY" FREEZING

Tumor	Tumor type	Tissue used for inoculation	Tumor generation	Number of animals		Takes		Average lag	
				B. & F.	Ordinary	B. & F., per cent	Ordinary, per cent	B. & F.	Ordinary
P1679	Lymphoid leukemia	Subcut. mass	8	6	6	100	100	16.5	16.5
P1643	Myeloid leukemia	Subcut. mass	6	6	6	0	0		

DISCUSSION

The experiments of Simpson (7) described above show that tissues frozen by immersion in isopentane chilled to or below $-100°$ C. are more severely damaged than those frozen in isopentane at or above $-60°$ C. Our fast method consisted of immersion of tissues in isopentane at about $-75°$ C. (freezing time 1 to 2 seconds). On the basis of Simpson's results, such tissues should be on the border between the zones of greater and lesser damage. Our results check closely with this, most of the tumors when frozen by the fast method having showed slightly greater damage than when frozen by the slow method. The two exceptions (tumor E0771 and L946AII) can perhaps be explained by the fact that the isopentane would be expected to act more rapidly at high temperatures than at low in producing chemical and physical changes, and hence would work in the opposite direction from the freezing effect. In any case some difference in the reaction of different tumors must be assumed.

The results obtained by Breedis (1) are in essential accord with those described above. He reported increased damage when a test tube containing a leukemic cell suspension at 0° C. was rapidly transferred to ether at −45° C. (freezing time 12 seconds). This slightly higher temperature for the dividing line between the zones of greater and lesser damage is most probably due to differences in the condition of the experiment; *e.g.,* use of cell suspensions, use of leukemias only, and separation of the cells from the freezing fluid by glass.

Mider and Morton's results, so far as they go, emphasize the importance of distinguishing between cells frozen as a suspension and *en masse.* There is a strong suggestion that the border line temperature between the zones of greater and lesser damage is considerably higher for the former than for the latter. Their method of freezing, because of the interposition of glass between the cells and the chilling medium, was apparently enough slower than ours, even though the temperature of the medium was the same, so that cells frozen *en masse* were not brought down into the more lethal zone, although cells frozen as a suspension apparently were.

While the rate of freezing separating the two zones, and the effect on the position of the boundary of varying the conditions of freezing other than rate are only as yet partly known, the existence of the two zones, one of greater damage with faster freezing, one of lesser damage with slower freezing, is indicated by all four sets of experiments. With still faster freezing it is possible that tissues can be brought down into a third zone where damage is at an absolute minimum (5, 7).

An extension of our results with isopentane chilled in liquid nitrogen to temperatures below −75° C. would be most desirable, but is not contemplated owing to difficulties in obtaining the necessary supplies.

SUMMARY

1. Immersion of small pieces of transplantable tumor in isopentane or freon 11 at room temperature for 30 minutes caused no reduction in the percentage of takes.

2. Most transplantable tumors frozen by direct immersion in isopentane at −75° C. (freezing time 1 to 2 seconds) were more severely damaged than the same tissues frozen by slower methods.

3. Freezing by the simple method of corking a small piece of tissue in a 1 cc. sterile vial and dropping it directly on dry ice in a thermos jug probably gives as satisfactory preservation as more elaborate methods.

4. Some (slowly growing?) tumors will not survive any method of freezing so far tried.

The writers are indebted to Dr. Lloyd Law for providing several of the leukemias.

REFERENCES

1. Breedis, C. The Action of Extreme Cold on Leukemic Cells of Mice. J. Exper. Med., **76**:221-240. 1942.
2. Breedis, C., Barnes, W. A., and Furth, J. Effect of Rate of Freezing on the Transmitting Agent of Neoplasms of Mice. Proc. Soc. Exper. Biol. & Med., **36**:220-224. 1937.
3. Breedis, C., and Furth, J. The Feasibility of Preserving Neoplastic Cells in the Frozen State. Science, **88**:531-532. 1938.
4. Chang, M., and Walton, A. The Effects of Low Temperature and Acclimatization on the Respiratory Activity and Survival of Ram Spermatozoa. Proc. Roy. Soc., London, s. B, **129**:517-527. 1940.
5. Luyet, B. J., and Gehenio, P. M. Life and Death at Low Temperatures. Normandy, Missouri: Biodynamica. 1940.
6. Mider, G. B., and Morton, J. J. The Effect of Freezing *in Vitro* on Some Transplantable Mammalian Tumors and on Normal Rat Skin. Am. J. Cancer, **35**:502-509. 1939.
7. Simpson, W. L. An Experimental Analysis of the Altmann Technic of Freezing-Drying. Anat. Rec., **80**:173-189. 1941.

A Rapid Test for Tumor Growth Inhibitors

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Investigations of yeasts and mold extracts by this test were carried out in cooperation with Dr. J. C. Keresztesy,* Dr. J. E. Little,* and Dr. J .W. Foster *

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The following report deals with a rapid test for tumor growth inhibitors. Studying the influence of various factors on tumor growth we needed a standardized method that would permit the detection of tumor growth inhibitors on a small number of animals in a short experimental period. Reviewing the literature we did not find such a method.

A certain degree of standardization was achieved in a test described by Fleisher and Loeb (6) in 1914. The growth rate of a transplanted adenocarcinoma of the mouse was determined after 4 days' treatment (1 intravenous injection on 4 successive days) by palpation or by weighing of the tumor. In the palpation method drawings of the tumors were made at the start of the experiment and compared with drawings made on the 3rd and 4th day of treatment. In the second test the final weight of the tumors was compared with the initial weight (calculated on the basis of drawings made with the aid of weighed models), and the difference expressed the degree of growth in 4 days. With these two tests the authors examined a large number of substances and found, for instance, that casein, colloidal copper, or hirudin retarded tumor growth.

MacFadyen and Murphy (13) elaborated a method for detecting tumor growth inhibitors on transplanted tumors with the aid of multiple inoculations. When they transplanted, into the same animal, one untreated tumor fragment and one that had previously been dipped for a definite period into a solution containing inhibitory substances, the latter failed to grow or grew less than the former. The use of one animal for both grafts reduced the influence of variations in host susceptibility but the necessity for application of the test material directly to the transplant seriously limits the usefulness of this method.

In extensive studies on chemotherapy Boyland (1–4) tested the action of many compounds on the growth of grafted and primary tumors and attempted (3) to express quantitatively and statistically the effect of inhibiting substances.

In the experiments with grafted tumors (M.C.D.B.I.), treatment was started on the day of transplantation. The tumors were measured twice weekly with calipers and the growth in groups of 5 to 15 treated mice compared with that in untreated mice. For evaluation of the result the t test was applied and the probability values were calculated. Apparently this tumor had such a regular growth rate that it was possible to measure differences of 15 per cent in growth, which were significant even in small groups of unmatched tumors.

In the experiments with primary tumors, the effect of a compound was measured on the growth rate of 4 single neoplasms. Each treated tumor was used as its own control, in such a way that the growth rate during a period preceding treatment was compared with the growth rate during treatment. Here it must be assumed that each individual primary tumor had a linear growth rate during the period of observation (up to 8 weeks).

In our studies on the growth of spontaneous tumors, however, wide fluctuations in the growth rate of individual tumors were observed. Without any treatment at all some, after a period of vigorous growth, showed slow or even completely arrested growth. Furthermore, the long time period needed for Boyland's test on grafted and primary tumors is disadvantageous.

Quantitative studies of the growth of untreated transplanted tumors have been made by Chambers and Scott (5), Woglom (19), Mayneord (14), Schrek (15–18), and others. A review of the literature and a critical discussion of the results may be found in Schrek's publications. He stresses the need for additional investigations and the establishment of a standardized technic. Schrek's quantitative studies indicate that there is linear growth in most tumors, but that the latent period (number of days between inoculation and development of a palpable nodule) and the growth rate may vary in individual transplants of the same tumor as well as in those from different ones. These observations suggest that factors influencing tumor growth can be studied on individual tumors if the

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latent period and growth rate of each tumor are determined prior to the treatment, or by selection of tumors of a fixed size at a fixed time after transplantation. The latter method would eliminate the need of studying latent period and growth rate before treatment.

Lewisohn and his associates (7–11) in this laboratory, who have studied the fate of spontaneous mammary adenocarcinomas in mice under the influence of various extracts, reported that 30 per cent of the treated tumors in mice fed normal diet, and 60 per cent in those fed a polished rice diet, regressed after 4 to 6 weeks of daily intravenous injections of yeast extract. Mere reduction in size, frequently observed after 1 to 2 weeks of treatment, was found not to be dependable, for fluctuations in untreated controls, after a simple biopsy, were rather high; neither temporary reduction, retardation of the growth rate, nor temporary arrest of growth was necessarily due to the treatment.

These experiments have the advantage of direct therapeutic action on a spontaneous tumor, but the disadvantages are obvious: The experiments are time-consuming, an ample supply of animals is not available, and the material is not sufficiently homogeneous in respect to general conditions and age of the animals, or the location, size, and type of the tumor, for quantitative studies.

In the following paragraph the method is described and a critical evaluation of the experimental conditions offered.[1] Furthermore, we present the results obtained with this test.

METHOD

The growth rate of sarcoma 180 is used as an indicator and the inhibition of tumor growth is judged by comparing tumor sizes and tumor weights in treated groups with those in untreated ones. At the start of the experiment 7 to 10 days after transplantation groups with the same number of mice are matched as to their tumor sizes (1.0 ± 0.2 cm. in diameter). When such matched groups are injected intravenously with saline solution twice a day for 2 consecutive days the tumor sizes and weights in these mice will agree after a period of 48 hours. But in similar groups treated instead with an extract containing tumor growth inhibitors the sarcomas will fail to grow or will grow more slowly and their terminal sizes and weights will differ considerably from those of tumors in the control groups injected with a saline solution.

[1] Details of the statistical evaluations are omitted from this publication; the data are available upon request.

Technic

Female mice of 18 to 20 gm. body weight, of the same stock and age, are injected with sarcoma 180 by the trocar method. As soon as the majority of the tumors reach a size of approximately 1 cm. in diameter, at from 7 to 10 days after inoculation, they are selected for the main experiment, the smaller or larger ones being used for preliminary tests. The final matching and grouping of the selected tumors is done in the following way: One is taken for group 1, the mouse is marked, the tumor is measured through its two perpendicular diameters with a pair of calipers, and a freehand approximation of its shape is drawn. The resulting area is measured in square inches with a planimeter to three decimal places, with uncertainty of less than one in the second place. Then a second tumor, which matches the first in size as closely as possible, is similarly marked for group 2 and so on to group *n*, until there are at least 7 tumors in group 1 and 7 matched tumors in each of the other groups. Though group 1 may not always have 7 tumors of exactly the same size, the average size for each of the several groups should always be the same. To lessen the error of selection, we proceed from group 1 to group *n* for the first tumor, from group *n* to group 1 for the second, and so on. After *n* groups have been selected, each mouse of a group is injected intravenously twice a day for 2 consecutive days with the preparation in question. The time intervals between the 4 injections should be as nearly equal as possible. After 48 hours the tumors are measured again and their outlines drawn. The animals are killed with ether, the tumors are removed, freed from surrounding tissue, and their weights determined in a weighing bottle on a balance with a sensitivity of 1 mgm.

The following paragraph describes in detail, with charts and statistical evaluations, one example from the series of experiments presented in Tables I and II.

Seventy-five female Rockland mice 18 to 20 gm. in weight and 4 months old were injected Apr. 10, 1942, with sarcoma 180, passage MP 15. On Apr. 18, 8 days after transplantation, 7 groups of 7 mice each were selected and their tumors matched. Group 67 was injected intravenously with 0.1 cc. of yeast extract used as a standard preparation, corresponding to 5.1 mgm. solids, twice daily for 2 consecutive days. Group 72 was injected simultaneously with 0.1 cc. saline intravenously. The other 5 groups served for testing unknown preparations. On Apr. 20, after 47 hours, the tumors were measured again and their outlines drawn as described. The animals were then killed with ether, the tumors removed and weighed, and the group means for size and weight calculated.

Female "Rockland" mice transplanted with Sarcoma 180 on April 10, 1942, passage MP 15
Four intravenous injections (2 on April 18, 1942 and 2 on April 19, 1942)

	with Standard preparation (S_3) 0.1 cc. = 5.1 mg. Group 67			with Saline solution 0.1 cc. Group 72		
Marking of mice	April 18 Initial size (sq. in.)	April 20 Terminal size (sq. in.)	April 20 Terminal weight (mg.)	April 18 Initial size (sq. in.)	April 20 Terminal size (sq. in.)	April 20 Terminal weight (mg.)
Front right	0.15	0.15	195	0.14	0.27	570
Front left	0.15	0.16	240	0.15	0.25	600
Hind right	0.15	0.14	190	0.15	0.23	720
Hind left	0.17	0.23	410	0.16	0.16	320
Both front	0.18	0.10	160	0.16	0.24	752
Both hind	0.16	0.16	310	0.14	0.29	660
Front right Hind left	0.13	0.17	270	0.13	0.30	850
Mean (σ of the mean)	0.156 (0.005)	0.159 (0.014)	254 (32)	0.147 (0.005)	0.249 (0.017)	639 (64)

FIG. 1.—Effect of a standard preparation on tumor growth.

At the start of the experiment the group means for the size of untreated tumors were 0.147 and for the treated ones 0.156 sq. in.; after 47 hours the group means for the untreated tumors were 0.249 sq. in. and for the treated ones 0.159 sq. in. The group means for weights were 639 mgm. for untreated, and 254 mgm. for the treated tumors. The probability ratio corresponding to the difference of the means is 4.1 for terminal size and 5.4 for terminal weight.

GROWTH OF UNTREATED MATCHED GROUPS

As the degree of inhibition of tumor growth is expressed by the difference between tumor sizes (and weights) in treated and untreated groups, the behavior of untreated groups had to be investigated first. The following questions were studied:

How reliable is the matching of tumors as judged by mean initial sizes?

It was found on 926 animals with 65 pairs of groups, that the mean initial sizes agreed with a mean error of 5.4 ± 0.5 per cent (Fig. 2).

How do such matched groups agree after 48 hours of growth as judged by mean terminal sizes and mean terminal weights?

Such groups of tumor mice, matched as to their initial sizes, agreed after 48 hours of growth with a mean error of 9.5 ± 2.6 per cent as judged by their mean terminal sizes and with a mean error of 7.6 ± 1.9 per cent as judged by their mean terminal weights.

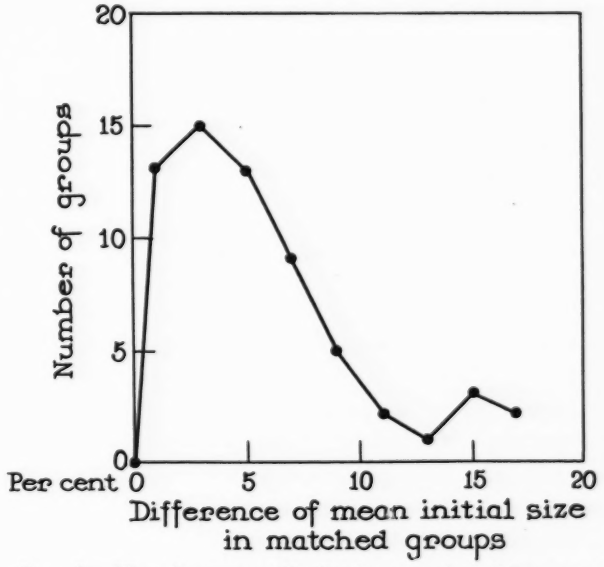

FIG. 2.—The frequency distribution of the difference of mean initial size in matched groups, expressed in percentages.

Has the initial tumor size any influence on the growth rate of the tumor in 48 hours, as judged by terminal size and terminal weight?

The correlation coefficients of initial size to terminal size and terminal weight are sufficiently large to indicate the importance of this effect. Hence it is desirable that all animals of any one group be matched as closely as possible in order to reduce the scatter of terminal size and terminal weight values within that group. The size of 1.0 ± 0.2 cm. in diameter was chosen because at this period (7 to 10 days after transplantation) this size indicates a vigorous growth of the transplant with but few regressive changes in the tumor.

Growth of Matched Groups of Mice Treated with Standard Preparations

After the conditions of growth of matched untreated groups were examined, the behavior of such matched groups under the influence of treatment with standard preparations was studied.

Influence of Standard Preparations in One Fixed Dose

Tables I and II demonstrate the effect of two standard preparations in one fixed dosage on the growth rate of matched tumors as judged by mean terminal sizes and mean terminal weights. In Table I the mean values for 7 groups in 7 different experiments for the treated animals are 0.147 sq. in. and 253 mgm.; for the corresponding matched control groups, 0.253 sq. in. and 674 mgm. In Table II the mean values for 9 groups in 9 different experiments are, for the treated ones, 0.139 sq. in. and 239 mgm.; for the corresponding untreated ones, 0.254 sq. in. and 616 mgm.

From these values it is evident that tumor growth is decidedly inhibited by the intravenous injection twice daily for 2 consecutive days of a standard yeast preparation. Since the corresponding mean values for the two standard preparations are in reasonable agreement (last line in Tables I and II) it follows that the two standards, prepared at different times, are comparable

Table I: Effect of Standard Preparation S3 in Fixed Dosage on Terminal Size and Terminal Weight

	Groups Treated With							
	Standard preparation S3, 0.1 cc. = 5.10 mgm.				Saline solution, 0.1 cc.			
Number of mice in each group	Group No.	Mean initial size, sq. in.	Mean terminal size, sq. in.	Mean terminal weight, mgm.	Group No.	Mean initial size, sq. in.	Mean terminal size, sq. in.	Mean terminal weight, mgm.
7	14/60	0.147	0.113	244.3	14/65	0.161	0.266	798.1
7	11/35	0.149	0.156	380.3	11/40	0.167	0.263	830.0
7	12/44	0.150	0.151	248.3	12/49	0.127	0.229	674.6
7	19/97	0.153	0.144	203.1	19/103	0.163	0.266	617.7
7	15/67	0.156	0.159	253.6	15/72	0.147	0.249	638.9
7	20/104	0.157	0.154	238.6	20/110	0.161	0.256	584.6
8	17/84	0.165	0.150	201.3	17/86	0.179	0.245	576.9
	Mean	0.154	0.147	253.0		0.158	0.253	674.0

Table II: Effect of Standard Preparation S2 in Fixed Dosage on Terminal Size and Terminal Weight

	Groups Treated With							
	Standard preparation S2, 0.1 cc. = 5.25 mgm.				Saline solution, 0.1 cc.			
Number of mice in each group	Group No.	Mean initial size, sq. in.	Mean terminal size, sq. in.	Mean terminal weight, mgm.	Group No.	Mean initial size, sq. in.	Mean terminal size, sq. in.	Mean terminal weight, mgm.
7	27/174	0.154	1.143	198.6	27/180	0.179	0.247	528.6
7	24/136	0.159	0.121	220.0	24/138	0.173	0.229	524.3
7	14/59	0.161	0.124	221.4	14/65	0.161	0.266	798.1
7	25/153	0.161	0.170	202.9	25/159	0.161	0.260	565.7
6	16/75	0.163	0.135	174.7	16/81	0.173	0.242	550.3
7	24c/146	0.173	0.157	277.9	24c/148	0.186	0.266	581.4
7	23/127	0.180	0.130	242.9	23/135	0.187	0.277	670.0
6	26/165	0.183	0.133	218.3	26/171	0.190	0.227	585.0
7	13/52	0.186	0.140	396.3	13/58	0.209	0.276	741.1
	Mean	0.169	0.139	239.0		0.180	0.254	616.0

in their activity. The difference of mean terminal sizes between the untreated and treated groups is 0.106 sq. in. in the series of Table I and 0.115 sq. in. in the series of Table II, which agree to ±4 per cent of their median; the difference of mean terminal weights between the untreated and treated groups is 421 mgm. in the series of Table I and 377 mgm. in the series of Table II, which agree to ±5 per cent of their median.

We calculated furthermore the coefficients of variation for the treated and untreated groups in these experiments. In the series of Table I this coefficient for the treated groups was 9.7 per cent for terminal size and 15.1 per cent for terminal weight; for the untreated ones it was 11.7 per cent for terminal size and 17.1 per cent for terminal weight. In the series of Table II the coefficient of variation for the treated ones was 11.3 per cent for terminal size and 16.6 per cent for terminal weight; for the untreated ones it was 10.0 per cent for terminal size and 16.3 per cent for terminal weight. It may be concluded, therefore, that the variability among the several groups of similarly treated animals was no greater than among the corresponding controls, and additional experiments with two other standard preparations, not tabulated in this article, support this conclusion.

The preparation of a standard yeast extract and its effect on spontaneous mammary carcinomas in mice have been described in previous papers. It has been found stable in activity for 2 years, when kept sterile on ice. The single dose injected was 0.1 cc. for each of the two standards used in the experiments of Tables I and II, corresponding to 5.1 and 5.25 mgm. solids respectively.

Influence of a Standard Preparation in Varying Doses

In a series of experiments the influence of varying doses of a standard preparation on the terminal size and terminal weight of groups of tumor mice was studied. Fig. 3, in which the control group is regarded as having been treated at zero dosage, graphically analyzes the result of such an experiment. A regression is indicated that may be sufficiently linear for a dose response equation if the control group is omitted. Otherwise, it is curvilinear with convexity toward the D axis.

A subsequent paper on quantitative determination of the effect of tumor growth inhibitors will deal with a mathematical analysis of terminal size and terminal weight values, obtained by varying the dose of a standard preparation.

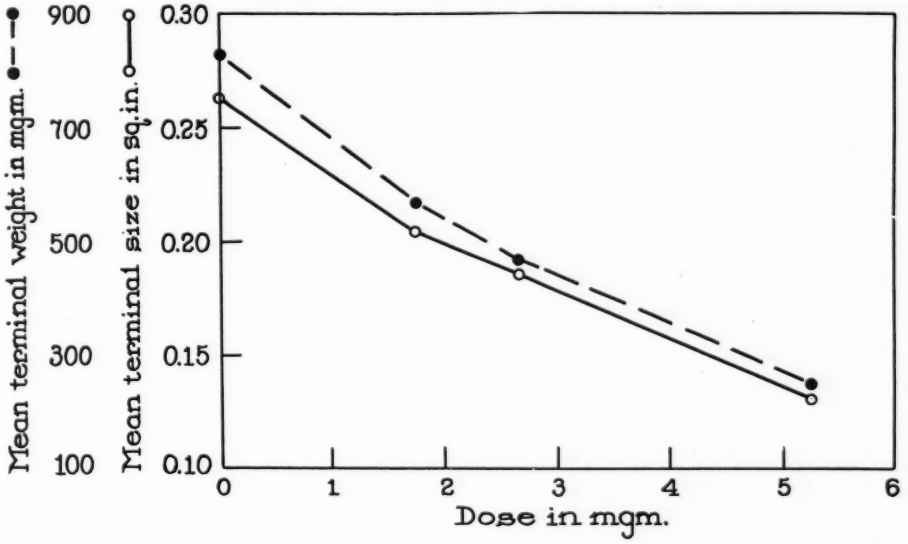

Fig. 3.—Effect of standard preparation S2 in varying doses on terminal size and terminal weight.

Influence of a Standard Preparation in Divided Doses

Having found that two daily injections of standard preparation in the dosage mentioned above gave a sufficient response in terminal size and terminal weight for a group of 7 to 10 mice, we next attempted to determine whether one daily injection, corresponding to the same daily total, would be adequate. In two experiments, 76 mice were divided into 8 matched groups. In 2 of these (19 animals), which were injected once daily, the terminal weight was 431 mgm.; in 2 (19 animals) injected twice a day, 299 mgm.; and in 4 control groups (38 animals), 563 mgm.

Furthermore, we tested whether dividing the total daily dose into 4 or 6 injections would change the

response, judged by terminal size or terminal weight, as compared with 2 injections. In two experiments 38 mice were divided into 4 matched groups; for 2 groups (19 animals) that were injected twice a day the terminal weight was 881 mgm.; for 2 (19 animals) that were injected 4 times a day it was 531 mgm. In two other experiments 72 mice were divided into 8 matched groups; for 2 groups (18 animals) injected twice a day the terminal weight was 466 mgm.; for 2 groups (18 animals) injected 6 times a day the terminal weight was 508 mgm.; for 4 control groups (36 animals) the terminal weight was 830 mgm.

These experiments indicate that one daily injection has less inhibitory effect than two, and the response is not sufficient to permit the detection and eventual quantitative measurement of inhibitors in a favorable range on a small number of animals. Four daily injections increased the effect definitely over two, and six daily injections caused inhibition comparable to two injections and less than four injections. These results suggest that a certain minimum dose at the single injection and repeated daily applications of this minimum are required for a sufficient inhibition of tumor growth. For reasons of convenience two daily injections were chosen.

RESULTS

With this method we have tested a large number of factors for possible inhibiting properties and have selected a number of experiments that may answer questions concerning its usefulness.

Influence of Extracts of Various Molds in Pure Culture and Brewer's Yeasts on Tumor Growth

During the past year we were confronted with a distinct drop in active substances in the yeast extract, caused by changes in the brewing process to which we have referred in previous papers (11, 12). Accordingly we tried different methods of extraction and studied the preparations with this test and, in addition, examined extracts from various brewer's yeasts and molds for inhibiting properties.

In Table III the results of experiments with 46 extracts are summarized. The testing of these extracts was performed according to the technic described above but for brevity's sake the inhibition of tumor growth is expressed in terminal weights only. Fourteen different molds (Nos. 1 to 14, Table III) in pure culture were examined in the form of extracts made as was the standard preparation and brought to the same final concentration so that equal amounts of solids were injected. In all fourteen experiments the tumor weights for animals treated with standard preparation were low, whereas the corresponding values for the groups treated with extracts of molds were considerably higher and similar to those for the controls.

In the next thirteen experiments (Nos. 15 to 28, Table III) 13 extracts of brewer's yeasts were tested. Though these were made in the same way as the standard preparation, and again the same amounts of solids were injected, none inhibited tumor growth so well as the standard; indeed, only one of them (No. 21) exerted, in one experiment, a distinct effect. Experiments 26 and 27 are especially to be mentioned. Here the extracts were made of yeasts from the brewery from which potent extracts had been obtained prior to the changes in the brewing process. The loss of potency, first observed in the treatment of spontaneous mammary carcinoma of mice, is apparent again in the results of this test.

In the next series of experiments the problem was studied whether the same yeast, obtained at varying stages of the brewing process (after 4, 6, 7, or 8 days of brewing) might yield extracts of different activity. However, none of these 6 extracts (Nos. 28 to 33) showed an activity comparable to that of the standard preparation, though the 4 day old culture (No. 31) was more active than the others.

In subsequent experiments we tried to determine whether changes in our extraction procedure (pH, temperature, time of extraction) might increase the yield of active agent. All these extracts were prepared from a 7 day old brewer's yeast. Extracts 34 and 35 were derived from the same source but prior to changes in the brewing process, and extraction was carried out at 80° C. for 7 minutes whereas the temperature for a standard preparation is 100° C. These two were tested after having been kept in an ice box for 2 years and were still fully active, but again all the other 11 extracts of yeast from the same source contained either distinctly less or hardly any activity compared to the standard preparation. The best yield was obtained by short watery extraction at 80° C.; extraction at lower or higher temperatures appeared to be less advantageous. The effect of heating is apparent in experiments 36, 37, and 38, the yield being much less after 3 minutes of boiling than at 80° C. initial heating. Similar results were obtained in experiments 44, 45, and 46.

Thus none of the molds tested showed any appreciable activity when extracted and injected in the same dose as the standard preparation, nor did a number of brewer's yeasts contain inhibitors in amounts comparable with that in the standard preparation. The use of 4 day old cultures of brewer's yeasts, and extraction at 80° C. initial heating, apparently increased the inhibitory activity.

Testing Fractions of Extracts and Various Pure Substances

A series of experiments [2] was carried out in which chemical fractions were tested and their chemical characterization was attempted. Furthermore a number of crystalline substances were examined for inhibiting properties. The results are reproduced in Table IV.

Table III: Effect of Extracts of Various Molds and Brewer's Yeasts * on Tumor Growth

Serial No.	Material under test,	Mean terminal tumor weight in groups treated with: mgm.	Standard yeast extract, mgm.	Saline solution, mgm.
1	*Zygopichia californica*	671	224	674
2	*Aspergillus wentii*	746	224	674
3	*Debaryomyces guillier-mondii*	640	224	674
4	*Torulospora delbruckii*	650	221	798
5	*Saccharomyces fragilis*	738	221	798
6	*Candida triadis*	587	174	587
7	*Rhizopus nigricans*	571	174	587
8	*Candida stellatoidea*	639	174	587
9	*Penicillium notatum*	943	308	—
10	*Torulopsis dattila*	754	308	—
11	Extract X	673	230	—
12	*Schwanniomyces occi-dentalis*	612	230	—
13	*Mycoderma lafarii*	602	236	—
14	Yeast strain R.	549	330	—
15	Yeast 20-40 F.	818	298	—
16	Regular beer yeast B.	509	203	613
17	Ale yeast B.	491	203	613
18	India pale ale yeast B.	564	273	634
19	India pale ale yeast B.; methanol precipitate at 95 per cent	447	203	556
20	Regular beer yeast S.	500	236	—
21	Regular beer yeast E.	298	207	537
22	Regular beer yeast E.	495	225	567
23	Regular beer yeast E.	518	234	514
24	Regular beer yeast E.	572	261	610
25	Dark beer yeast R.	446	198	529
26	Regular beer yeast R.	476	203	613
27	Regular beer yeast R.	403	203	613
28	Regular beer yeast R., 7 days' brewing	618	243	670
29	Regular beer yeast R., 7 days' brewing; methanol precipitate at 95 per cent	505	203	556
30	Regular beer yeast R., 7 days' brewing; autolyzed	655	243	670
31	Regular beer yeast R., 4 days' brewing	393	207	537
32	Regular beer yeast R., 6 days' brewing	528	207	537
33	Regular beer yeast R., 8 days' brewing	562	207	537
34	Regular beer yeast R., 1940, 80° C., 7 minutes	240	207	537
35	Regular beer yeast R., 1940, 80° C., 7 minutes	213	225	567
36	Regular beer yeast R., 1942, 80° C., initial heating	307	225	567
37	Regular beer yeast R., 1942, initial boiling	348	225	567
38	Regular beer yeast R., 1942, 3 minutes' boiling	450	225	567
39	Regular beer yeast R., 1942, 55° C., 5 minutes at pH 5	431	230	624
40	Regular beer yeast R., 1942, 80° C., 5 minutes at pH 5	384	230	624
41	Regular beer yeast R., 1942, 55° C., 20 minutes at pH 5	397	201	554
42	Regular beer yeast R., 1942, 65° C., 20 minutes at pH 5	393	201	554
43	Regular beer yeast R., 1942, 65° C., 5 minutes at pH 5	394	201	554
44	Regular beer yeast R., 1942, 80° C., 5 minutes at pH 4.5	294	245	516
45	Regular beer yeast R., 1942, 80° C., 5 minutes at pH 4.5	287	201	554
46	Regular beer yeast R., 1942, 100° C., 25 minutes at pH 4.5	416	201	554

* We are greatly indebted to the various breweries for supplying the yeasts.

Experiment 1 (Table IV) illustrates one example in which the solubility of the active principle in methanol was studied. After it had been found that the active principle in a standard preparation (S2) is precipitated by 95 per cent methanol at a temperature of 2° C., the activity of the precipitates thrown down by various lower concentrations was investigated. With concentrations below 75 per cent and above 80 per cent the precipitates were found to be inactive. The activity of the precipitate obtained with methanol between 75 and 80 per cent concentration at a temperature of 2° C. is shown in No. 1 (Table IV). One-tenth cubic centimeter, corresponding to 0.14 mgm. solids, injected intravenously inhibited tumor growth to an extent

[2] Some of these investigations were performed with the other members of our group.

comparable to 0.1 cc., corresponding to 5.25 mgm. solids in the standard preparation from which this precipitate was derived. It might also be mentioned that this active precipitate is atoxic when administered intravenously in this dose to mice, whereas the inactive counterparts, especially the material soluble in methanol at 95 per cent final concentration, are rather toxic.

In experiments 2 to 12 (Table IV) the results of testing a few crystalline substances upon tumor growth are shown. The dose injected is indicated in the table. In experiment 8 a vitamin mixture (composition and dose indicated in the footnote to Table IV) was tested. None of these substances caused any inhibition.

The experiments just mentioned illustrate that this test can be used to investigate the activity of samples obtained during chemical fractionation, and it has been employed also in the search for inhibitory activity in various pure substances.

Table IV: Effect of a Chemical Fraction of Standard Preparation S2 and of Crystalline Substances on Tumor Growth

Serial No.	Dose, mgm.	Material under test,	Mean terminal tumor weight in groups treated with: mgm.	Standard yeast extract, mgm.	Saline solution, mgm.
1	0.14	Methanol precipitate of S2	219	243	670
2	1.00	Thiamin	519	205	561
3	0.04	Riboflavin	547	205	561
4	2.00	Pyridoxin	506	205	561
5	2.00	Pantothenic acid	500	205	561
6	2.00	Nicotinic acid	506	205	561
7	2.00	*p*-Aminobenzoic acid	685	323	650
8	*	Vitamin mixture	497	181	507
9	2.00	Ascorbic acid	727	323	650
10	1.00	Histamine	458	—	410
11	2.50	Ethylenediamine	586	261	610
12	10.00	Methionine	578	168	444

* 0.1 cc. of the vitamin mixture contained 50γ of thiamin, 10γ of riboflavin, 50γ of pyridoxin, 50γ of pantothenic acid, 50γ of nicotinic acid, 50γ of *p*-aminobenzoic acid, 64γ of choline, and 2.5γ of biotin. The single dose injected was 0.2 cc.

The Influence of Dietary Factors on Tumor Growth

With the aid of the test it was possible to show that the feeding of polished rice or pearled barley, among other grains, increased the inhibiting effect of the standard preparation, for if mice were fed these diets instead of a normal one 1.75 mgm. of the standard preparation inhibited tumor growth to an extent comparable to 5.25 mgm. of the same standard preparation. Experiments proved that this increased inhibitory effect is not caused by dietary deficiency, but that it might be explained by the presence of inhibiting factors in these grains. Extracts prepared from polished rice or pearled barley inhibited tumor growth to a similar extent as the standard preparation when injected intravenously into mice kept on a normal diet. These experiments have been presented in detail in a recent paper (12).

DISCUSSION

Having analyzed the conditions under which inhibition of tumor growth can be tested, and described and illustrated its measurement, we proceed now to amplify some of the factors involved.

As the degree of inhibition is judged by differences between treated and untreated groups, increase in the size of the tumor must be sufficiently large in the untreated control groups during the experimental period. The shorter this period, the fewer irregularities within any one group may be expected. Sarcoma 180 was chosen because its growth rate is rapid enough, in most cases, to permit termination of the experiment after 48 hours.[3] Whether other transplanted tumors would be as suitable, or even better, for testing purposes cannot yet be answered. Sufficiently rapid and regular growth rate are prerequisites.

As was evident from the coefficient of correlation of initial size with terminal size and with terminal weight, the extent of growth in 48 hours depends on the size of the tumor at the start of the experiment. Consequently, in order to improve the agreement of terminal size and terminal weight data, either a selection of a uniform initial size for each individual of a group, or a mathematical adjustment for the variability in initial size is suggested.

[3] In those few experiments where the growth rate of sarcoma 180 over a period of 48 hours was inadequate, the experiments were extended over an additional 24 hours and used for qualitative tests of activity.

At least two injections every 24 hours are needed, each of one-half the total amount, since one undivided injection of the same amount does not inhibit tumor growth sufficiently to permit the detection and eventual quantitative measurement of inhibitors in favorable range on a small number of animals. The material must be injected intravenously, as subcutaneous, intramuscular, or intraperitoneal administration is ineffective.

The experiments with extracts of various molds and brewer's yeasts indicate that this test can be applied in the search for tumor growth inhibitors in such preparations. They demonstrate also that toxic extracts do not inhibit tumor growth in this test. The negative results with yeast extracts after changes had been made in the brewing process, in contrast to the positive results of the standard preparations, made previous to these changes, provide a further indication that this test is useful for tracing tumor growth inhibitors.

The results on the fractionation of the standard preparation show that it is possible with this test to differentiate fractions containing tumor growth inhibitors from inactive toxic ones.

The experiments on the influence of dietary factors illustrate that the test can be applied also to studies of these factors, for it helped to disclose the presence of inhibitors in various grains and to find new sources of raw materials from which inhibitors could be extracted.

As for the relation between inhibition of tumor growth and therapeutic action no conclusions should be drawn concerning the ultimate fate of sarcoma 180 in treated animals, for the test is used to detect inhibitors over a period of 48 hours only. The same reservations apply to spontaneous tumors. In a number of instances a parallelism was found between the results of the rapid test and therapeutic results with both sarcoma 180 and spontaneous mammary carcinomas in mice. For instance the standard preparation, which inhibited tumor growth in the test, was active against sarcoma 180 and against spontaneous tumors as well. Dietary factors such as polished rice or pearled barley increased the effect of the standard preparation in both instances, and extracts of pearled barley were equally effective both in the test and in the treatment of spontaneous tumors. At the present time, however, we cannot draw any definite conclusions from a positive test as to the action of an extract on spontaneous tumors. The standard preparations effected complete regressions in 30 per cent of spontaneous mammary cancers in mice kept on a normal diet. On the other hand, some of the extracts and fractions that gave figures in the test practically identical with those for standard preparations, showed either a complete absence of total regressions or an incidence much lower than 30 per cent when extracts and fractions were tested on spontaneous malignant tumors. It seems important to mention that none of the various extracts and fractions that showed a negative test ever effected the complete regression of spontaneous tumors.

Further comparative studies between the inhibition of tumor growth and therapeutic action on spontaneous malignant tumors are warranted.

SUMMARY

1. A rapid test is described for the detection of tumor growth inhibitors. Inhibition is judged by comparing tumor sizes and weights in treated and untreated groups of mice bearing sarcoma 180, after a period of 48 hours of growth. The groups are matched as to initial size of the tumors. The results achieved by the method are presented.

2. The practical application of the test is illustrated by several examples.

We are indebted to Dr. Franklin Hollander for his valuable advice and his great help in the statistical evaluations of the results.

REFERENCES

1. Boyland, E. Experiments on the Chemotherapy of Cancer. 1. The Effect of Certain Anti-Bacterial Substances and Related Compounds. Biochem. J., **32**:1207-1213. 1938.
2. Boyland, E., and Boyland, M. E. Experiments on the Chemotherapy of Cancer. III. The Independence of Tissue Respiration and Glycolysis, and the Growth Rate of Tumours. Biochem. J., **33**:618-621. 1939.
3. Boyland, E. Experiments on the Chemotherapy of Cancer. 4. Further Experiments with Aldehydes and Their Derivatives. Biochem. J., **34**:1196-1201. 1940.
4. Boyland, E. Experiments on the Chemotherapy of Cancer. 5. The Effect of Muscle Extract and Aliphatic Bases. Biochem. J., **35**:1283-1288. 1941.
5. Chambers, H., and Scott, G. M. Variations in the Growth of the Jensen Rat Sarcoma and the Influence of Technique. J. Path. & Bact., **33**:553-561. 1930.
6. Fleisher, M. S., and Loeb, L. The Influence of Various Substances on the Growth of Mouse Carcinoma. J. Exper. Med., **20**:503-521. 1914.
7. Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., and Laszlo, D. Effect of Intravenous Injections of Yeast Extract on Spontaneous Breast Adenocarcinomas in Mice. Proc. Soc. Exper. Biol. & Med., **43**:558-561. 1940.
8. Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., and Laszlo, D. Treatment of Spontaneous Breast Adenocarcinomas in Mice with Extracts of Spleen or Yeast. Am. J. Path., **17**:251-260. 1941.
9. Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., Laszlo, D., and Bloch, K. Action of Yeast Extract on Transplanted and Spontaneous Malignant Tumors in Mice. Cancer Research, **1**:799-806. 1941.
10. Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R,. Laszlo, D., and Bloch, K. Prevention of Tumor Growth (Carcinoma 2163) by Intravenous Injections of Yeast and Vitamins. Science, **94**:70-71. 1941.

11. Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., Laszlo, D., and Dische, Z. Influence of a Polished Rice Diet upon Spontaneous Mammary Cancers in Mice Treated with Yeast Extract. Cancer Research, **2**:818-822. 1942.

12. Lewisohn, R., Laszlo, D., Leuchtenberger, C., Leuchtenberger, R., and Dische, Z. Presence in Various Grains of Factors Inhibiting Tumor Growth. Proc. Soc. Exper. Biol. & Med., **52**:269-272. 1943.

13. MacFadyen, D. A., and Murphy, Jas. B. A Method for the Study of Induced Interference with Transplantable Tissue Growth. J. Exper. Med., **70**:461-473. 1939.

14. Mayneord, W. V. On a Law of Growth of Jensen's Rat Sarcoma. Am. J. Cancer, **16**:841-846. 1932.

15. Schrek, R. A Quantitative Study of the Growth of the Walker Rat Tumor and the Flexner-Jobling Rat Carcinoma. Am. J. Cancer, **24**:807-822. 1935.

16. Schrek, R. Further Quantitative Methods for the Study of Transplantable Tumors. The Growth of R39 Sarcoma and Brown-Pearce Carcinoma. Am. J. Cancer, **28**:345-363. 1936.

17. Schrek, R. The Effect of the Size of Inoculum on the Growth of Transplantable Rat Tumors. Am. J. Cancer, **28**:364-371. 1936.

18. Schrek, R. Permanent and Transient (Fortuitous) Variations of the Growth Components of Transplantable Rat Tumors. Am. J. Cancer, **28**:372-388. 1936.

19. Woglom, Wm. H. Regression of the Jensen Rat Sarcoma. J. Cancer Research, **9**:171-189. 1925.

Diplochromosomes in a Goldfish Tumor

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It has been demonstrated recently that the predominant mitotic figures in a number of mouse cancers contain diplochromosomes, which are made up of twice as many chromonemas as are chromosomes of embryonic or regenerating adult tissues and hence are twice as large (1). In lesser frequency are mitotic figures with chromosomes of normal size and those with polytene chromosomes of 4 or 8 times normal size. The mouse cancers studied included the fibrosarcoma XG of Selle, Brindley, and Spies (7), which arose from transplanted liver cells of C3H mice bearing methylcholanthrene-induced tumors elsewhere on the body; tumor 10591A of the Roscoe B. Jackson Memorial Laboratory; Crocker mouse sarcoma 180; a spontaneous mammary carcinoma; and 6 primary methylcholanthrene-induced sarcomas of C3H mice.

The presence of diplochromosomes in tumors would explain many puzzling reports, such as those on nuclei of double the normal volume (2), on an increased number of nucleoli (5), on false reduction divisions with the apparent pairing of homologous chromosomes (8), and on the enlarged chromosomes themselves (6). The frequency of such reports (1) makes it advisable to investigate the chromosomes of various neoplasms.

A study has been made of the chromosomes in an adenoma or adenocarcinoma of the ovary in a goldfish. The tumor was spontaneous in origin and had been in evidence for over a year as a slowly increasing massive distention of the abdomen. The tissue was pressed out in acetocarmine on slides after fixation in Carnoy's acetic-alcohol solution, and the mitotic figures were compared under oil immersion with those of nongerminal cells in a young normal ovary and with those of regenerating adult skin similarly prepared. Since goldfish chromosomes are rather small rods or short V's, and the diploid number approaches 90, very accurate counts were difficult to make. Occasionally hopeless jumbles of chromosomes were left unanalyzed, and sometimes chromatids were mistaken for whole chromosomes. For each mitotic figure, the width and average length of the chromosomes were computed with the aid of camera lucida drawings. Then, considering the metaphase chromosome as made up of a pair of cylinders (chromatids), the average chromosome volume for each mitosis was calculated. The volume of each figure or nucleus was reckoned as the average of the volumes of a short cylinder and an ellipsoid, after measurements had been made by means of the fine adjustment screw and a ruler beneath the mirror of the camera lucida. The results, incorporated in Table I, represent means of 10 figures for the normal, 10 for the tumor, and 3 for the stroma of the neoplasm. Standard deviations are included.

TABLE I: GOLDFISH METAPHASE FIGURES

	Normal	Tumor	Stroma
Chromosomes measured	81 ± 12	89 ± 12	92 ± 9
Volume of figure	$271 \pm 51\ \mu^3$	$387 \pm 90\ \mu^3$	$220 \pm 99\ \mu^3$
Chromatid radius	$0.16 \pm 0.02\ \mu$	$0.20 \pm 0.01\ \mu$	$0.14 \pm 0.01\ \mu$
Average length of chromosome	$0.96 \pm 0.09\ \mu$	$1.15 \pm 0.08\ \mu$	$0.94 \pm 0.16\ \mu$
Average volume of chromosome	$0.15 \pm 0.03\ \mu^3$	$0.29 \pm 0.03\ \mu^3$	$0.12 \pm 0.00\ \mu^3$

While the metaphase chromosomes of the normal tissue have a mean volume of 0.15 μ^3, the chromosomes of the tumor cells average 0.29 μ^3, or approximately double the normal. Figs. 1 and 2 illustrate representative normal and cancerous metaphases. The larger size of the tumor chromosomes is manifested not only in an increased width but also in a greater length. Accompanying the increase in chromosome size in the neoplastic tissue of the goldfish there is an increase of roughly one-half in the volumes of the metaphase figures and the resting nuclei, as well as an increase of the maximum nucleolar number in the resting nuclei from 4 in the case of the normal tissue to 8 in the case of the cancerous. This last fact is significant, for it means that the double size of the tumor chromosomes results from a doubled strand number, since the nucleoli are borne on specific organizers on certain chromosomes. With about the

* Fellow of The International Cancer Research Foundation.

same number of chromosomes in the normal and tumorous cells, the tumor chromosomes must be diplochromosomes to account for the doubled number of nucleoli.

These facts all parallel those found in cancers of mice (1), with the exceptions that: (a) In the mouse the diploid tumor nuclei are just double the volume of the diploid normal nuclei. (b) There are still more highly polytene chromosomes in the mouse tumors, although none larger than diplochromosomes were found in the goldfish. (c) There is more polyploidy in the mouse cancers; only one polyploid mitosis was seen in the goldfish adenoma, a tetraploid anaphase with diplochromosomes. Like the mouse cancers, the goldfish neoplasm contained normal stroma cells with small nuclei and chromosomes of normal size.

FIG. 1

FIG. 2

FIG. 1.—Metaphase of mitosis in a goldfish skin wound. Ninety-one chromosomes with an average volume of 0.15 μ^3.

FIG. 2.—Metaphase of mitosis in a goldfish ovarian tumor. Ninety-one chromosomes with an average volume of 0.31 μ^3.

The diplochromosomes of the tumor are probably formed by an endomitotic cycle in which the centromeres either are not reduplicated or, if reduplicated, are not separated. This endomitosis must accompany carcinogenesis, possibly as a secondary effect of the carcinogen. Repeated endomitosis without centromere division brings about more highly polytene chromosomes and another increase in nuclear volume. Apparently the frequency of repeated endomitosis in a tumor is proportional to the degree of malignancy, to judge from such papers as that of Greenough (4), who found that great variability in nuclear size is associated with high malignancy in a cancer. This goldfish neoplasm was of low malignancy, for it had been growing a long time, no invasion of other organs was evident, the malignant cells were chiefly in single-layered epithelium and stroma was abundant, mitotic figures were none too frequent, and cytoplasmic basophilia was not pronounced. It has been shown that the intensity of cytoplasmic staining is proportional to the degree of malignancy (3), and that the staining is probably determined by the concentration of ribonucleic acid in the cytoplasm (1). That the goldfish adenoma had no chromosomes more highly polytene than diplochromosomes is in accord with its low malignancy. The significance of the presence of diplochromosomes and more highly polytene chromosomes in cancerous tissues is not clear, and it must be remembered that such chromosomes are normally present in certain tissues, especially of insects.

SUMMARY

The chromosomes in an ovarian tumor of a goldfish had an average volume double that of the chromosomes in a healing skin wound and in nongerminal cells of a normal ovary. The maximum number of nucleoli was 4 in the normal nuclei and 8 in the cancerous. Hence the chromosomes of the goldfish tumor must be regarded as diplochromosomes.

REFERENCES

1. BIESELE, J. J., POYNER, H., and PAINTER, T. S. Nuclear Phenomena in Mouse Cancers. University of Texas Publication No. 4243. Austin. 1942.
2. EHRICH, W. E. Nuclear Sizes in Growth Disturbances. With Special Reference to the Tumor Cell Nucleus. Am. J. M. Sc., **192**:772-790. 1936.
3. EVANS, N., BARNES, R. W., and BROWN, A. F. Carcinoma of the Prostate. Correlation between the Histologic Observations and the Clinical Course. Arch. Path., **34**:473-483. 1942.
4. GREENOUGH, R. B. Varying Degrees of Malignancy in Cancer of the Breast. J. Cancer Research, **9**:453-463. 1925.
5. VON HAAM, E., and ALEXANDER, H. G. Cytological Studies of Malignant Tumors. Am. J. Clin. Path., **6**:394-414. 1936.
6. LUDFORD, R. J. The Behaviour of the Malignant and Non-Malignant Cells of Transplantable Tumours in Tissue Cultures. Arch. f. exper. Zellforsch., **14**:42-55. 1933.
7. SELLE, W. A., BRINDLEY, P., and SPIES, J. W. The Production of Tumors by Transplantation of Normally Appearing Liver Cells from Animals Previously Injected with Methylcholanthrene. Cancer Research, **1**:618-619. 1941.
8. WINGE, O. Zytologische Untersuchungen über die Natur maligner Tumoren. II. Teerkarzinome bei Mäusen. Ztschr. f. Zellforsch. u. mikr. Anat., **10**:683-735. 1930.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BADGER, G. M., COOK, J. W., HEWETT, C. L., KENNAWAY, E. L., KENNAWAY, MRS. N. M., and MARTIN, R. H. [Chester Beatty Research Inst., Royal Cancer Hosp. (Free), London, England] THE PRODUCTION OF CANCER BY PURE HYDROCARBONS. VI. Proc. Roy. Soc., London, s. B, 131:170-182. 1942.

A series of eight 5-alkyl derivatives of 1,2-benzanthracene (methyl, ethyl, propyl, *iso*-propyl, butyl, amyl, hexyl, heptyl) was tested for cancer-producing activity. The higher members of the series showed progressive diminution of carcinogenic power upon the skin, and those having more than three carbon atoms in the side chain did not produce sarcoma. Tests upon several 10-substituted benzanthracenes are recorded; the 10-cyano- and 10-β-hydroxyethyl compounds produce both epitheliomas and sarcomas. The results given by several new derivatives of 3,4-benzphenanthrene (2-*n*-propyl-, 1-*iso*-propyl-, 2-ethyl-, 2-formyl-, 2-acetyl-) confirm earlier findings in showing (1) that substitution in position 2, and to a less degree in position 1, promotes carcinogenic action, and (2) that most of these compounds are deficient in sarcoma-producing power. 1,2,3,4-Tetramethylphenanthrene, which has a weak but quite distinct property of producing tumors of the skin, is one of the simplest carcinogenic compounds known. In its molecular structure it forms a connecting link between the carcinogenic hydrocarbons of the 1,2-benzanthracene and 3,4-benzphenanthrene groups, and is also related to 1,2-dimethylchrysene which is one of the two homologues of chrysene known to be carcinogenic. 3,4,5,6-Dibenzfluorene differs from the 1,2,3,4-, 1,2,5,6-, and 1,2,7,8- compounds in showing no action upon the skin. Further tests were made of the carcinogenic power of 1,2,5,6-dibenzcarbazole in view of its considerable growth inhibitory power. The relation between two azonaphthalenes (1,2′- and 2,2′-) and the diaminodinaphthyls and dibenzcarbazoles that may be obtained from them is discussed in regard to the production of tumors of the liver. Some data are given of the occurrence of multiple and remote tumors (of the skin, lung, stomach, and ureter) especially in mice receiving certain classes of compounds (2,3,4- substituted phenanthrenes, 1,2,5,6-dibenzcarbazole, and 1,2,5,6-dibenzacridine). Apparently the production of numerous adenomas of the lung can occur under treatment with a compound, 1,2′-diamino-1′,2-dinaphthyl, which has produced no tumors at the sites of application or injection.—E. L. K.

CAMERON, G., KOPAC, M. J., and CHAMBERS, R. [Washington Square Coll. of Arts and Science, New York Univ., New York, N. Y.] NEOPLASM STUDIES. IX. THE EFFECTS IN TISSUE CULTURE OF N,N-DIMETHYL-p-PHENYLENEDIAMINE ON RAT LIVER TUMORS INDUCED BY p-DIMETHYLAMINOAZOBENZENE. Cancer Research, 3:281-289. 1943.

Normal glandular epithelium of the rat liver, grown in tissue culture, is sensitive to N,N-dimethyl-*p*-phenylenediamine in concentrations as low as 0.0001 *M*, while equivalent effects are produced on ductal epithelium with concentrations of 0.002 *M* or higher. The resistance of ductal epithelium from normal liver is slightly less than that from tumors. Glandular liver epithelium, whether normal or carcinomatous, is sensitive to the action of N,N-dimethyl-*p*-phenylenediamine, the normal being slightly more sensitive. Concentrations of sodium sulfite ranging from 0.001 to 0.005 *M* retard or prevent the oxidation of N,N-dimethyl-*p*-phenylenediamine which, in the reduced state, is nontoxic. Completely oxidized solutions are also nontoxic. The toxicity of N,N-dimethyl-*p*-phenylenediamine is probably confined to the intermediate stages in its oxidation. The resistance of normal ductal epithelium to N,N-dimethyl-*p*-phenylenediamine explains the preponderance of cholangioma production in livers following the feeding to rats of *p*-dimethylaminoazobenzene plus nonprotective diets. The occasional presence of glandular cell tumors is believed to be the result either of the detoxification of N,N-dimethyl-*p*-phenylenediamine in the liver (to its diacetyl derivatives) or of its maintenance in the reduced, nontoxic state. Liver glandular epithelium can survive only when the oxidizable concentration of this compound is low (0.0001 *M*) or when in the form of a diamine. High concentrations (0.005 *M*), under autoxidizable conditions, are probably responsible for cirrhotic conditions in the liver following the feeding of *p*-dimethylaminoazobenzene to rats.—Authors' abstract.

KOPAC, M. J., CAMERON, G., and CHAMBERS, R. [Washington Square Coll. of Arts and Science, New York Univ., New York, N. Y.] NEOPLASM STUDIES. X. THE EFFECTS IN TISSUE CULTURE OF SOME SPLIT PRODUCTS OF p-DIMETHYLAMINOAZOBENZENE ON RAT LIVER TUMORS. Cancer Research, 3:290-292. 1943.

Several split products of *p*-dimethylaminoazobenzene were tested on normal liver and rat liver tumors grown in tissue culture. Aniline hydrochloride or aniline sulfate, also *p*-aminophenol hydrochloride at concentrations of 0.001 *M*, were nontoxic to both glandular and ductal epithelia. Either *p*-phenylenediamine or *p*-phenylenediamine hydrochloride, under autoxidizable conditions, at

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0.001 *M* was toxic to all cells except those that form intracellular crystals (possible oxidation polymers of the diamine). Intracellular crystal formation could be blocked by sodium sulfite and, in such cases, *p*-phenylenediamine was nontoxic to epithelial cells and wandering cells.

None of the split products mentioned above was found to be so toxic as N,N-dimethyl-*p*-phenylenediamine which, at concentrations of 0.001 *M*, destroyed normal or neoplastic glandular epithelium. On the other hand, ductal epithelium (normal or neoplastic) at this concentration survived and grew.—Authors' abstract.

CHAMBERS, R., CAMERON, G., and KOPAC, M. J. [Washington Square Coll. of Arts and Science, New York Univ., New York, N. Y.] NEOPLASM STUDIES. XI. THE EFFECTS IN TISSUE CULTURES OF N,N,N′,N′-TETRAMETHYL-o-PHENYLENEDIAMINE AND OTHER COMPOUNDS ON MALIGNANT LYMPH NODES. Cancer Research, 3:293-295. 1943.

The compounds tested on tissue cultures were several found by Kensler (unpublished data) to inhibit the respiration of malignant lymphoid tissue but not that of normal lymph nodes. In tissue culture it was found that some of the compounds acted in a similar way on the viability of outgrowing cells while others did not. The minimum effective concentration of sodium malonate (0.005 *M*), 8-hydroxyquinoline (0.2 saturation in serum), and rotenone (0.1 saturation in serum) indiscriminately destroyed all cells. N,N-Dimethyl-*p*-phenylenediamine, at 0.001 *M*, destroyed all lymphoid cells irrespective of their source but did not affect the macrophages and fibroblasts, which were destroyed at 0.004 *M*. In a third group were N,N,N′,N′-tetramethyl-*o*-phenylenediamine and N,N,N′N′-tetraethyl-*o*-phenylenediamine. The tetramethyl compound was about 7 times more toxic to the lymphoid cells of lymphosarcomatous and leukemic nodes (0.001 *M*) than to those of normal lymph nodes (0.0075 *M*), human and rat. Lymphoid cells from the nodes of Hodgkin's disease were almost but not quite as sensitive as those from the other malignant nodes. The tetraethyl compound had a potency of about half that of the tetramethyl compound.

It is not possible from present data to decide upon the actual identity of the lymphoid cells of the normal and malignant tissues studied. The majority of those appearing in cultures of normal lymph nodes are of the small round cell type. The cells of similar appearance in the malignant nodes may not be precisely those of the normal nodes. This is suggested by the greater variety in shape of the cells in malignant cultures, and their tendency to form irregularly lobate instead of the characteristic relatively minute and narrow pseudopodia of normal lymphocytes. They may be of an immature type, which raises the possibility that immaturity *per se* is the causative factor in their excessive sensitivity. However, the over-all difference in sensitivity of the lymphoid cells from malignant and normal sources to tetraethyl- and tetramethyl-*o*-phenylenediamine is sufficiently pronounced to be significant.—Authors' abstract.

EDWARDS, J. E., and DALTON, A. J. [Nat. Cancer Inst., Bethesda, Md.] INDUCTION OF CIRRHOSIS OF THE LIVER AND OF HEPATOMAS IN MICE WITH CARBON TETRACHLORIDE. J. Nat. Cancer Inst., 3:19-41. 1942.

Repeated oral administration of carbon tetrachloride in olive oil to mice of strains C3H, A, C, and Y, autopsied at 1 year of age or less, resulted in a hepatoma incidence of 88.2%. The tumors were well differentiated and resembled other hepatomas of the mouse and rat both morphologically and chemically. Successful transplantation of the tumor has been achieved. Repeated oral administrations of olive oil alone failed to raise the incidence of hepatoma above that of the spontaneous variety.

Single or limited doses of carbon tetrachloride in concentrations sufficient to cause hepatic necrosis failed to induce tumors. However, if the same total amount of carbon tetrachloride was given in divided doses over a period of 2 months, hepatomas developed in 71% of the mice.

Repeated administration of hepatotoxic doses of carbon tetrachloride resulted in cirrhosis of the liver within a period of about 6 weeks. The histological picture following withdrawal of carbon tetrachloride after the development of cirrhosis is described.—F. L. H.

Hormones

VARGAS, L., Jr. [Carnegie Inst. of Washington, Baltimore, Md.] EXPERIMENTAL FIBROIDS IN HYPOPHYSECTOMIZED FEMALE GUINEA PIGS. Cancer Research, 3:309-317. 1943.

In the experimental fibroid reaction induced by estrogen in the guinea pig, it was of interest to investigate the possible participation of the hypophysis. Twenty-one hypophysectomized adult female guinea pigs were treated by subcutaneous implantation of pellets of estradiol (5 to 10 mgm.). Another group of 15 nonhypophysectomized estrogen-treated animals was kept as controls. Because ovariectomy enhances the degree of the estrogen-induced blastomatogenesis, almost all the females were castrated.

Serial sections from 14 of the 21 hypophysectomized animals demonstrated that the pituitary stalk and the pars tuberalis usually remained in the sella turcica. In all the cases but one, microscopic fragments of the pars intermedia were found upon high power study, although direct examination of the sella turcica with the binocular dissecting microscope had never shown such fragments.

The degree of mammary development also served as a control of the hypophysectomy, since when the extirpation of the anterior lobe was complete the mammary glands did not develop.

In regard to the blastomatogenesis, complete extirpation of the anterior lobe of the hyophysis had no influence on the experimental fibroid reaction in castrated adult female guinea pigs; neither the intensity, extent, nor distribution of the process was affected. Incomplete removal of the anterior lobe gave the same results except for a difference in the development of the mammary gland, since microscopic fragments of the pars anterior permitted mammary growth.

Thus the blastomatogenic effect of the estrogen is not exerted through the anterior lobe of the hypophysis.—Author's abstract.

Genetics

BURDETTE, W. J. [Yale Univ. Sch. of Med., New Haven, Conn.] THE INHERITANCE OF SUSCEPTIBILITY TO TUMORS INDUCED IN MICE. II. TUMORS INDUCED BY METHYLCHOLANTHRENE IN THE PROGENY OF C3H AND JK MICE. Cancer Research, 3:318-320. 1943.

The reciprocal crosses of C3H and JK parents were made, and the progeny were injected subcutaneously with 1 mgm. of methylcholanthrene. With conditions similar to those present when the parental strains were studied, average median and mean appearance times for tumors in F_1 mice were intermediate to those of the C3H and JK strains. No evidence for sex linkage of susceptibility factors or for extrachromosomal influence was found. The average survival time of the hybrids with tumors was greater than that of either the C3H or JK parents. The results obtained are compatible with the theory of the presence of more than one gene for susceptibility to induced tumors, at least one of which is dominant and at least one of which is recessive.—Author's abstract.

Physical Factors

SPENCER, R. R., and MELROY, M. B. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF CARCINOGENS ON SMALL ORGANISMS. IV. EXPOSURE OF BACTERIA TO HIGH TEMPERATURES. J. Nat. Cancer Inst., 3:1-5. 1942.

A simple technic is described for the regular transfer of bacterial cultures, which seems to minimize the possibility of air-borne contaminations.

Continuous exposure of *Streptococcus hemolyticus* (N. Y. No. 5 strain) to a temperature of 42° C. caused death after the fifth or seventh consecutive transfer. Rhythmic exposure to the same temperature did not interfere with growth and survival. Relatively similar results were obtained with *Eberthella typhosa* at 45° C.

The process of species adjustment to an unfavorable environment as a mechanism in carcinogenesis is discussed. —F. L. H.

Radiation

MITCHELL, J. S. [Univ. of Cambridge, England] DISTURBANCE OF NUCLEIC ACID METABOLISM PRODUCED BY THERAPEUTIC DOSES OF X AND GAMMA RADIATIONS. PART I: METHODS OF INVESTIGATION. Brit. J. Exper. Path., 23:285-295. 1942.

The experimental methods are described. Ultraviolet photomicrography was mainly used, but measurements of ultraviolet absorption and fluorescence spectra, and histochemical tests, were also made. Unirradiated and irradiated fixed, unstained biopsy specimens were photographed on the same microscopic field, and the degree of blackening at any point was measured photometrically. Maximum "optical staining" was obtained by use of the wave length 2,537 Å. From these data the effective concentration of the nucleic acids was deduced.—H. G. C.

MITCHELL, J. S. [Univ. of Cambridge, England] DISTURBANCE OF NUCLEIC ACID METABOLISM PRODUCED BY THERAPEUTIC DOSES OF X AND GAMMA RADIATIONS. PART II: ACCUMULATION OF PENTOSE NUCLEOTIDES IN CYTOPLASM AFTER IRRADIATION. Brit. J. Exper. Path., 23:296-309. 1942.

The results obtained by the study of biopsy specimens of 32 clinical cases treated with gamma or x-radiation are assembled and discussed.

An increase in the ultraviolet absorption of the cytoplasm of cells after irradiation was found in 11 out of 15 pairs of biopsy specimens from cases treated with gamma rays, and in 13 out of 17 cases treated with x-rays. This increased optical density was shown typically by proliferating and differentiating cells, but was almost absent in fully differentiated cells. No quantitative differences of behavior were found between tumor cells and homologous cells of adjacent normal and hyperplastic tissues.

There was no parallelism between the increased degree of ultraviolet absorption and the dose of radiation. For example, with single doses of gamma radiation ranging from 250 to 4,400 r, the optical change was constant. The dose rate, however, seemed important. The increase in cytoplasmic absorption was fairly constant for dose rates 1.0 to 444 r per minute but only low values were found with dose rates less than 0.9 r per minute.

Recovery from this metabolic disturbance in typical radiosensitive cases was complete after 24 hours, though wide variations in the recovery time were found. In exceptional cases the disturbance persisted unchanged for as long as 56 days.

For the interpretation of the observed phenomena many other technics were applied. Ultraviolet absorption spectra of substances accumulating after x-radiation are shown and the presence of adenine inferred. A small increase of fluorescence induced by radiation was observed. Histochemical tests suggested the presence of pentose, but not desoxypentose, in the cytoplasm of most cells after irradiation. Also, the diazo reaction, applied after benzoylation of sections, gave results consistent with the presence of adenine, small amounts of guanine, and perhaps uracil.

The general conclusion reached from these various studies is that irradiation causes the accumulation (often up to 3%) of pentose nucleotides, probably ribonucleotides containing adenine and some other unidentified chromophoric groups, in the cytoplasm, and that these products are manifested by increased ultraviolet absorption.—H. G. C.

MITCHELL, J. S. [Univ. of Cambridge, England] DISTURBANCE OF NUCLEIC ACID METABOLISM PRODUCED BY THERAPEUTIC DOSES OF X AND GAMMA RADIATIONS. PART III: INHIBITION OF SYNTHESIS OF THYMONUCLEIC ACID BY RADIATION. Brit. J. Exper. Path., 23:309-313. 1942.

Measurements by three different methods were made of the density of absorbing materials within the nuclei of normal and irradiated cells. In contrast to the increase of ribonucleotides found in the cytoplasm, no comparable increase of nucleic acid was found in the nucleus after irradiation.

These changes are explained as due to inhibition by the radiation of the process of reduction of ribonucleotides to desoxyribonucleotides in the nucleus.—H. G. C.

WARREN, S. [New England Deaconess Hosp., Boston, Mass.] THE DISTRIBUTION OF DOSES OF RADIOACTIVE PHOSPHORUS IN LEUKEMIC PATIENTS. Cancer Research, 3:334-336. 1943.

Radioactive phosphorus administered as Na_2HPO_4 to leukemic patients is absorbed and retained to varying degrees by different tissues of the body. The present data are based on 10 patients dead of advanced leukemia.

The amount of phosphorus retained tends to be greatest in those organs that show heavy infiltration by leukemic cells. Relatively large amounts are present in the liver, spleen, kidneys, and bone marrow. There is some excretion of radioactive phosphorus in the bile. The slowly metabolizing tissues, such as the brain and cartilage, contain but little phosphorus. The distribution of radioactive phosphorus in man is of approximately the same order as is found experimentally in rodents.—Author's abstract.

Biochemistry and Nutrition

ALBAUM, H. D., and POTTER, V. R. [Univ. of Wisconsin, Madison, Wis.] BIOCATALYSTS IN CANCER TISSUE. II. INHIBITION OF THE SUCCINOXIDASE SYSTEM BY TUMOR EXTRACTS. Cancer Research, 3:303-308. 1943.

Experiments have been carried out on an inhibitor from tumor tissues with a succinoxidase preparation from liver as a test system. The results of these experiments may be summarized as follows: Healthy tumor tissues contain no inhibitor. Necrotic tumor or healthy tumor after autolysis possesses such an inhibitor. Liver, after autolysis, also shows inhibitor activity. Pancreas, as well as crystalline trypsin, chymotrypsin, and ribonuclease, inhibits succinoxidase activity. The inhibitory action is not completely destroyed by heating, and it is suggested that the heat-stable inhibition may be due to SH compounds and to ribonuclease in certain tissues. It is concluded that succinoxidase assays on healthy tumor tissues would not involve inhibitor action and would therefore be completely valid.—Authors' abstract.

GREENSTEIN, J. P. [Nat. Cancer Inst., Bethesda, Md.] SULFHYDRYL GROUPS IN NORMAL AND TUMOROUS HEPATIC TISSUE EXTRACTS BEFORE AND AFTER ADDITION OF SALTS. J. Nat. Cancer Inst., 3:61-67. 1942.

The free sulfhydryl group content of hepatoma 31 transplants was about 24% lower than that of normal rat liver. The treatment of extracts of these tissues with a wide variety of salts increased the sulfhydryl groups in the hepatoma by 64% and in the liver by 133%. Halides were the most active in releasing sulfhydryl groups while sucrose and the ammonium salts and phosphates that were used were without action.

The sulfhydryl group content of the following tissues was determined before and after the addition of guanidine hydrochloride: normal rat, mouse, and rabbit liver, transplants of rat hepatoma 31, mouse hepatomas 587, 98/15, and A, regenerating rat liver, the livers of tumor-bearing rats and mice, and fetal rabbit livers. The increase in the sulfhydryl group content of mouse liver and hepatoma was greater than in the corresponding rat tissues. In mice, the values for sulfhydryl content were the same for normal liver, for liver of tumor-bearing animals, and for the different kinds of transplanted hepatomas. The sulfhydryl content of the rabbit liver was much greater than that of the rat. The content of mercaptan groups in the necrotic area of the hepatoma 31 transplants and in fetal rabbit liver was very small. The sulfhydryl content of regenerating rat liver was the same as that of normal rat liver. The livers of the tumor-bearing animals had the same mercaptan content as the livers of normal animals.—F. L. H.

GREENSTEIN, J. P., EDWARDS, J. E., ANDERVONT, H. B., and WHITE, J. [Nat. Cancer Inst., Bethesda, Md.] COMPARATIVE ENZYMATIC ACTIVITY OF TRANSPLANTED HEPATOMAS AND OF NORMAL, REGENERATING, AND FETAL LIVER. J. Nat. Cancer Inst., 3:7-17. 1942.

The activity of arginase, catalase, xanthine dehydrogenase, amylase, thymonucleodepolymerase, and of acid and alkaline phosphatase was determined in the following transplanted hepatomas: 98/15 in C3H mice, which arose spontaneously, 7A/77 in A mice, which arose by repeated injection of carbon tetrachloride, and 587 in A mice, which arose spontaneously. The data obtained were compared with earlier data on hepatoma 1 in I mice and on hepatoma A in C3H mice, both of which arose by injections of *o*-aminoazotoluene, and on transplants of hepatoma 31 originally induced by feeding *p*-dimethylaminoazobenzene. Data previously obtained with fetal rabbit liver, regenerating rat liver, and the normal liver of all these strains and species were used for comparison.

The conclusion is reached that a consideration of the enzymatic activity of tumors must take into account the following factors: (1) The particular enzyme system concerned; (2) the kind of tumor; (3) the species of animal; (4) the strain of animal; and (5) the activity of the normal tissue of origin.—F. L. H.

MINER, D. L., MILLER, J. A., BAUMANN, C. A., and RUSCH, H. P. [Univ. of Wisconsin, Madison, Wis.] THE EFFECT OF PYRIDOXIN AND OTHER B VITAMINS ON THE PRODUCTION OF LIVER CANCER WITH p-DIMETHYLAMINOAZOBENZENE. Cancer Research, 3:296-302. 1943.

Thirty-six groups of 15 rats each were fed highly purified diets containing *p*-dimethylaminoazobenzene and crystalline synthetic B vitamins. The dye was fed for 4 months and the livers inspected by laparotomy at 4 and 6 months. The incidence of tumors was low when moderate amounts of the vitamins were fed. These amounts, however, were adequate for the maintenance of adult rats for 6 months. When the levels of all the B vitamins were raised well above the amounts necessary for maintenance, the tumor incidence in the presence of the azo dye reached 66% at 6 months.

Under the conditions of the experiment the incidence of tumors due to *p*-dimethylaminoazobenzene was decidedly lowered when the level of pyridoxin in the diet was reduced, or when the vitamin was omitted entirely. Large amounts of pyridoxin fed to resistant rats tended to increase the incidence of tumors.

The addition of large amounts of riboflavin completely prevented the appearance of tumors in rats receiving only 12% of casein. At carcinogenic levels of the synthetic B vitamins the incidence of tumors was essentially the same with 12% as with 18% of casein in the diet.

The production of tumors appeared to be more difficult in animals raised to maturity on a diet consisting of fortified milk than in animals raised on a diet relatively low in the B vitamins.—Authors' abstract.

PIRIE, A. [Imperial Cancer Research Fund, London, England] A HYALURONIDASE AND A POLYSACCHARIDE FROM TUMOURS. Brit. J. Exper. Path., 23:277-284. 1942.

The presence of spreading factor in some human and animal tumors has been demonstrated by Duran-Reynals and Stewart and by Boyland and McClean. Since Chain and Duthie have shown that spreading factor is closely associated with hyaluronidase, Pirie used the test for enzyme activity in an examination of a series of animal tumors.

Tumor extracts were incubated with a polysaccharide obtained from Fujinami and Rous sarcomas and the degree of ensuing hydrolysis was estimated from the change in the amount of reducing substances which took place during incubation. It was found that a mouse sarcoma (No. 37), a mouse mammary cancer (No. 63), a mouse skin cancer (No. 2146), the Jensen rat sarcoma, Dael's guinea pig sarcoma, and the slowly growing filterable fibrosarcoma of fowls (Mill Hill 1) all exhibit a weak hyaluronidase activity. The Shope papilloma, Fujinami myxosarcoma, and Rous sarcoma all gave inactive extracts. Viscous extracts of the Fujinami and Rous sarcomas were easily clarified and made limpid by testis hyaluronidase but no increase in titer of virus was observed. The viscous polysaccharide of tumors was isolated and studied. The preparations contained varying amounts of copper, which acts as a catalyst in the reduction in viscosity of the polysaccharide by ascorbic acid.—W. E. G.

SHARPLESS, G. R., and SABOL, M. [Henry Ford Hosp., Detroit, Mich.] CHOLINE AND PYRIDOXINE AS FACTORS IN PREVENTION OF EPITHELIAL HYPERPLASIA IN THE FORESTOMACH OF RATS FED WHITE FLOUR. J. Nutrition, 25:113-117. 1943.

Sixty-three per cent of rats fed a basal diet of white flour, calcium lactate, sodium chloride, ferric citrate, butterfat, thiamin, riboflavin, and viosterol developed one or more gastric lesions. The incidence of lesions remained the same when the basal diet was supplemented with pyridoxin and fell to 31% when supplemented with choline hydrochloride. Feeding both choline and pyridoxin, or choline, pyridoxin, and calcium pantothenate reduced the incidence of lesions to 14% and caused slightly better growth.—F. L. H.

WHITE, J., and EDWARDS, J. E. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF SUPPLEMENTARY METHIONINE OR CHOLINE PLUS CYSTINE ON THE INCIDENCE OF p-DIMETHYLAMINOAZOBENZENE-INDUCED HEPATIC TUMORS IN THE RAT. J. Nat. Cancer Inst., 3:43-59. 1942.

Rats on a basal low protein-high fat diet (White-Jackson) had a low incidence of cirrhosis of the liver. Supplementation of the basal diet with 0.5% *l*-cystine raised the incidence of this lesion to 70%. No hepatic tumors developed in either group.

Supplementation of the basal diet with 0.5% *l*-cystine and 0.06% *p*-dimethylaminoazobenzene gave a 96.5% incidence of primary malignant hepatic tumors in the rats. On this same diet without the cystine the tumor incidence fell to 60%. Cirrhosis of the liver was present in both groups but was more extensive in the group receiving supplementary cystine.

Supplementation of the basal diet of one group of rats with 0.06% *p*-dimethylaminoazobenzene, 0.5% *l*-cystine, and 0.2% choline and of another group with 0.06% *p*-dimethylaminoazobenzene and 0.5% methionine resulted in a 90.0% incidence of primary malignant tumors of the liver in each group. The animals of these two groups did not show any extensive cirrhosis of the liver but did show an almost constant increase in intralobular connective tissue or reticulum.

The individual tumors and hepatic lesions are described and illustrated with photomicrographs.—F. L. H.

Immunology

GROSS, L. [Christ Hosp., Cincinnati, Ohio] INTRADERMAL IMMUNIZATION OF C3H MICE AGAINST A SARCOMA THAT ORIGINATED IN AN ANIMAL OF THE SAME LINE. Cancer Research, 3:326-333. 1943.

One hundred and fifteen mice of the C3H inbred line were inoculated intradermally with doses varying from 0.01 cc. to 0.03 cc. of a 20% cell suspension of a sarcoma that had been originally induced by methylcholanthrene in an animal of the same line. The resulting intradermal tumors regressed spontaneously in 21 animals. Repeated intradermal reinoculations with the same sarcoma of most of the animals that recovered were, with few exceptions, unsuccessful. Five of those that recovered were also reinoculated subcutaneously with the sarcoma, and 4 of them proved resistant. All control animals, inoculated simultaneously by the same routes and with equal amounts of the same tumor suspensions, developed sarcomas. Since all animals, including the one in which the tumor originated, were of the same inbred line, experiments reported in this study suggest that immunity resulting from intradermal immunization is directed specifically against the tumor used for inoculation, and does not depend upon genetic differences between the animal inoculated and the one that originally produced the neoplasm. The theoretical significance of these observations is discussed and the importance of accurate dosage in the study of experimental tumor immunity is emphasized.—Author's abstract.

Miscellaneous

CARR, J. G. [Inst. of Animal Genetics, Edinburgh, Scotland] THE ABSENCE OF A SEASONAL INFLUENCE UPON THE ROUS NO. 1 SARCOMA IN YOUNG CHICKS. Brit. J. Exper. Path., 23:339-342. 1942.

Between 1935 and 1939, 971 chicks 6 to 9 weeks old were injected intramuscularly with cell-free material of the Rous tumor. An analysis of the results of the inoculations shows that seasons of the year have no influence on the susceptibility of the chick.—W. E. G.

Comparative Oncology

CAMPBELL, J. A. [Nat. Inst. for Med. Research, London, England] THE INCIDENCE OF PRIMARY LUNG TUMOURS IN A MIXED STRAIN OF MICE. Brit. J. Exper. Path., 23:191-201. 1942.

An account of the incidence of primary lung tumors in 2,225 mice of mixed stock, chiefly chocolate, fawn, and white, used in the author's experiments (abstracted in Cancer Research, **1**: 328. 1941; **2**: 579. 1942). The tumors are very rare before 10 months of age. Females fight less than males, and hence live longer and show a higher in-

cidence of lung tumors and of metastases. The material studied comprised 362 mice bearing primary lung tumors (90 control and 272 experimental); 41 of the former (46%) and 154 of the latter tumors (56%) were malignant. Metastasis occurred in 1 control and in 9 experimental mice (all female). The fawn mice appear to be somewhat more liable to lung tumors. Each experimental batch must be accompanied by a control batch, as the incidence varies in mice living 10 months or more (from 8.9% to 14.2% in controls, average 11.3%) and has declined in recent years. The 33 mammary tumors (2 of these in males) occurred in 2.5% of the control and 1.4% of the experimental mice; the author does not state in which series the tumors in males occurred.—E. L. K.

INNES, J. R. M. [Imperial Chemical (Pharmaceuticals) Ltd., Manchester, England] NEOPLASTIC DISEASES OF THE TESTIS IN ANIMALS. J. Path. & Bact., 54:485-498. 1942.

Fifty-two tumors of the testis were examined—1 from a calf, 2 from horses, and the remainder from dogs—and classified as follows: teratoma (1 case), seminoma (33 cases), benign interstitial cell tumor (1 case), malignant interstitial cell tumor (2 cases), and Sertoli cell tumor or tubular adenoma (15 cases). In addition there were 12 cases of nodular hyperplasia of interstitial cells.

Testicular tumors seem to occur more frequently in animals than in man. Evidence is given to show that the nodular hyperplasia of interstitial cells is not truly neoplastic, and that seminoma in dogs is of low grade malignancy as compared with the corresponding human tumor.

The paper is illustrated with four plates and includes an excellent bibliography and short review of the veterinary literature.—A. H.

OLCOTT, C. T., and PAPANICOLAOU, G. N. [Cornell Univ. Med. Coll., New York, N. Y.] STUDIES ON SPONTANEOUS TUMORS IN GUINEA PIGS. III. A CHONDROSARCOMA OF THE ILIAC BONE WITH METASTASIS TO THE MAMMARY REGION. Cancer Research, 3:321-325. 1943.

A spontaneous chondrosarcoma attached to the ilium and metastasizing to the mammary region of a senile female guinea pig is described.—Authors' abstract.

Clinical and Pathological Reports

Etiology

CARSON, W. J. [St. Luke's Hosp., Milwaukee, Wis.] TRAUMA AND MALIGNANCY. Am. J. Surg., 59:420-428. 1943.

During 30 years spent in a surgical clinic and pathological laboratory, the author has seen but two cases of sarcoma which seemed to fulfill the criteria of a sarcoma due to a single trauma.—H. G. W.

Multiple Tumors

MAJOR, J. W. [Vanderbilt Univ. Sch. of Med., Nashville, Tenn.] SIMULTANEOUS LEIOMYOSARCOMA OF THE UTERUS AND PAPILLARY CARCINOMA OF THE OVARY. Arch. Path., 35:115-120. 1943.

A case report.—H. G. W.

MATHÉ, C. P., and STEISS, C. F. [St. Mary's Hosp., San Francisco, Calif.] DOUBLE MALIGNANCY: HYPERNEPHROMA AND BASAL CELLED CARCINOMA OF NOSE. Am. J. Surg., 57:376-380. 1942.

A case report.—H. G. W.

STEIN, R. J. [Orleans County Memorial Hosp., Newport, Vt.] INTRACELLULAR INCLUSION BODIES IN CARCINOMA OF THE ADRENAL GLAND. A CASE OF MULTIPLE PRIMARY TUMORS. Am. J. Clin. Path., 12:630-634. 1942.

A case is described of triple primary carcinoma; namely, carcinoma of the breast, adenocarcinoma of the rectum, and carcinoma of the adrenal, of which the last alone showed numerous intracellular inclusion bodies.—H. G. W.

Diagnosis—General

SAPHIR, O. [Chicago, Ill.] PRECANCEROUS LESIONS. Proc. Inst. Med. Chicago, 13:435-442. 1941.

Various types of precancerous lesions are discussed, and the importance of careful assay of every diagnosis of precancerous lesion is emphasized. The lesions considered are endocervicitis, intestinal polyposis, papillary tumors of the bladder, chronic cystic mastitis, intracystic papilloma of the breast, leukoplakia, hyperkeratoses, and lesions due to irritants. It is advised that a healthy skepticism be exercised in interpreting these lesions. Analysis of several groups of statistics on precancerous lesions tends to discount the belief that a large number of so called precancerous lesions eventuate in cancer.—R. C. R.

Radiation—Diagnosis and Therapy

BUSCHKE, F., and CANTRIL, S. T. [Swedish Hosp., Seattle, Wash.] ROENTGENOTHERAPY OF CARCINOMA OF URINARY BLADDER. AN ANALYSIS OF 52 PATIENTS TREATED WITH 800 K.V. ROENTGENTHERAPY. J. Urol., 48:368-383. 1942.

An analysis of 52 cases of advanced carcinoma of the bladder treated with 800 kv. roentgen irradiation shows 7 of these cases to be cystoscopically free of disease for periods varying from 7 years (3 cases) to 6, 5, 2, and 1 year respectively.—H. G. W.

GARDNER, W. J., and NOSIK, W. A. [Cleveland Clinic, Cleveland, Ohio.] EXPERIENCES WITH ENCEPHALOGRAPHY IN CEREBELLAR TUMOR. Am. J. Roentgenol., 47:691-698. 1942.

The injection of air into the spinal subarachnoid space in cases of subtentorial tumor is universally condemned. At the Cleveland Clinic, encephalography is freely used in patients with brain tumor, although ventriculography is employed for subtentorial tumors. Encephalography was inadvertently carried out on 24 patients with subsequently verified cerebellar tumors. There were 3 unfavorable reactions with 1 death. Characteristically, the films showed absence of air in the ventricles, unusual prominence and curving of the supracallosal and singulate sulci, flattening of the pontine cisterns, and the presence of cerebellar herniation. The films do not localize the lesion but indicate that it is in the posterior fossa.—C. E. D.

GERSHON-COHEN, J., and SHAY, H. [Philadelphia, Pa.] CARCINOMA OF THE COLON. EARLY DIAGNOSIS WITH DOUBLE CONTRAST ENEMA. Penn. M. J., 44:462-466. 1941.

The roentgen methods of diagnosing cancer of the colon that depend upon the barium enema are satisfactory only when the lesions are large and well advanced.

The double contrast enema, however, makes possible the diagnosis of early lesions. The earliest stages of carcinoma were found in the absence of gastrointestinal symptoms. In a small group of cases, the only common finding preceding the symptoms was occult blood in the stools. The technic of the double contrast enema is described and several roentgenographic illustrations are presented.—J. L. M.

HARVEY, W. F. [Royal Coll. of Physicians' Laboratory, Edinburgh, Scotland] REVIEW OF IRRADIATION EFFECT ON CELLS AND TISSUES OF THE SKIN. Edinburgh M. J., 49:529-552. 1942.

After a review of the physical agents involved in the production of radiation effects, the histological changes are described and discussed in relation to the cell, connective tissue and blood vessels, epidermis and skin appendages—for such topics as radio-sterilization, radio-ulceration, radio-atrophy, radio-carcinoma, and xeroderma pigmentosum. So far as the mode of action of radiation is considered, stress is laid on the essentially destructive nature of the process, proliferation being regarded as a secondary reaction.

Two cases of roentgen carcinoma are referred to, the first showing an extremely early stage of squamous cell carcinoma, and the second the most advanced changes after a total duration of more than 30 years.

The paper is well illustrated (35 figures) and is provided with a useful bibliography.—A. H.

HOWES, W. E., and BERNSTEIN, L. [Brooklyn, N. Y.] METHODS USED AND RESULTS OBTAINED IN TREATMENT OF WIDESPREAD METASTASES SECONDARY TO MAMMARY CANCER. Radiology, 38:562-572. 1942.

Much can be accomplished by the palliative treatment of widespread metastases from breast cancer. Pain is often relieved, bone metastases may recalcify, skin metastases may be destroyed, visceral metastases, except those in the liver, may be improved, and useful life is often prolonged from one to several years. Data are presented on 124 cases, showing the distribution of metastases and the duration of life after the appearance of metastases. The technic of radiation therapy is described with charts, photographs, and illustrative roentgenograms. Eighty-three treated patients lived an average of 20 months as compared with an estimated 5 months' survival of untreated patients.—C. E. D.

ILL, E. A. [Newark, N. J.] TWENTY YEARS' EXPERIENCE IN THE TREATMENT OF CARCINOMA OF THE UTERINE CERVIX AND BREAST WITH RADIUM. J. M. Soc. New Jersey, 38:445-450. 1941.

During a period of 20 years the author treated approximately 1,000 patients with cancer of the cervix. About 30% remained tumor-free for at least 5 years. He considers radium an auxilliary to surgical treatment of mammary cancer, especially when it is impossible to remove large axillary metastases completely. Two examples of favorable results in cases of the latter type are cited.—M. J. E.

LENZ, M. [Presbyterian Hosp., New York, N. Y.] ROENTGEN THERAPY OF CANCER OF THE BREAST AND REGIONAL METASTASES: PREOPERATIVE AND NON-OPERATED CASES. Radiology, 38:686-697. 1942.

A study is presented of 82 patients with cancer of the breast who received roentgen therapy between 1933 and 1937 at the Presbyterian Hospital, New York. In 38 patients this treatment was followed by radical mastectomy. Tables are given showing the relationships between the size of the primary tumor, the presence of axillary metastases, the histological type, the gross and microscopic response to radiation, and the ultimate outcome. Patients whose tumors showed initial regression after irradiation survived longer than the others. Small tumors showed greater regression than large ones. Radiation produced greater regression in the primary tumors than in axillary metastases. The five year survival rate among the patients with axillary involvement (6 of 18) is higher than might be expected from surgical treatment alone.—C. E. D.

O'BRIEN, F. W. [Boston, Mass.] THE PLACE OF IRRADIATION IN THE TREATMENT OF CANCER OF THE BREAST. Radiology, 38:524-532. 1942.

The benefits to be derived from radiation therapy in inoperable recurrent or metastatic cancer of the breast are generally accepted. The role of radiation in primary operable cases is problematical. As yet, no convincing data support the theory that preoperative irradiation is useful but a plea is made for its fair trial. The author has found postoperative irradiation beneficial in cases with lymph node metastases. Roentgen castration of women who are still menstruating after the age of 50 might be warranted as a preventive of breast cancer.—C. E. D.

SCHWARTZ, C. W. [New York, N. Y.] TUMORS OF THE ACOUSTIC NERVE. FROM A ROENTGENOLOGICAL VIEWPOINT. Am. J. Roentgenol., 47:703-710. 1942.

Acoustic nerve tumors constitute about 9% of all intracranial new growths. They usually originate within the bony canal near the internal auditory meatus. Bone atrophy about the bony canal and in the petrous pyramid at the internal auditory meatus are the earliest roentgenological changes. Planigraphy is useful in making the meatus visible. A shift in the position of the pineal body may be of help. The differential diagnosis between acoustic neuroma and other subtentorial growths is discussed.—C. E. D.

SOILAND, A. [Los Angeles Tumor Inst., Los Angeles, Calif.] PREOPERATIVE IRRADIATION OF BREAST CANCER. Radiology, 38:537-539. 1942.

The author presents data showing a 67% five year survival in 24 cases of group I carcinoma of the breast treated by preoperative irradiation and a 54% survival in 53 cases of group II similarly treated. He believes this represents an improvement over the results of surgery alone. Practically identical survival rates were obtained in cases of operable cancer treated with radium implantation plus roentgen rays without surgery, while the results of roentgen therapy alone were inferior.—C. E. D.

WARREN, S. [Boston, Mass.] EFFECTS OF RADIATION ON NORMAL TISSUES. Arch. Path., 35:121-139. 1943.

Part of a general review, this section dealing with effects on the gonads and the nervous system.—H. G. W.

WEBER, H. M., and KIRKLIN, B. R. [Mayo Clinic, Rochester, Minn.] ROENTGENOLOGIC MANIFESTATIONS OF TUMORS OF THE SMALL INTESTINE. Am. J. Roentgenol., 47:243-253. 1942.

One hundred and eight cases of malignant tumor of the small intestine together with 41 cases of benign tumor were studied in an attempt to determine the accuracy of

roentgenologic diagnosis. In the 36 instances in which adequate examination of the small intestine was carried out, the presence and site of the lesion was determined in 32 and missed in only 4. The specific diagnosis of carcinoma was made in but 6 cases, the remainder being reported chiefly as obstructing or ulcerating lesions. A considerable number of diagnostic errors arose from difficulty in differentiating tumors of the first portion of the duodenum from benign ulcers. Lesions of the ileum are the most likely to be overlooked entirely. Nineteen roentgenograms and photographs are presented.—C. E. D.

YOUNG, B. R. [Temple Univ. Med. Sch., Philadelphia, Pa.] THE DEMONSTRATION OF TUMORS, NON-NEOPLASTIC DISEASE, AND FOREIGN BODIES IN THE NECK AND CHEST BY BODY SECTION ROENTGENOGRAPHY (PLANIGRAPHY). Pennsylvania M. J., 44:713-717. 1941.

By means of body section roentgenography, disease processes in the neck and chest may be demonstrated that are not made visible by any other method of roentgen examination. Because it eliminates the spine shadow in the neck, anteroposterior planigraphy enables the demonstration of soft tissues in this region, especially those of the larynx. Any change in shape of the airway due to trauma, severe infections, or carcinoma is revealed by planigraphy, though such a demonstration is impossible by conventional roentgen studies. Tumors in the chest are delineated despite the presence of pleural fluid or dense lung tissue in the neighborhood of the neoplasm. Not only is a tumor made visible by anteroposterior planigraphy but its depth is determined, and thus aspiration biopsy is possible. Lung cavities that are not seen by ordinary film examinations are clearly demonstrated by planigraphy. This technic is as reliable in ruling out lung tumors or cavities of any appreciable size as it is in establishing the presence of either of these two conditions. The paper is well illustrated.—J. L. M.

Skin and Subcutaneous Tissues

DRIVER, J. R., and MacVICAR, D. N. [Western Reserve Sch. of Med., Cleveland, Ohio] CUTANEOUS MELANOMAS. A CLINICAL STUDY OF SIXTY CASES. J.A.M.A., 121: 413-419. 1943.

A study of 60 cases, in 36.7% of which the primary growth was on the lower extremities; in 41.7% on the head; in 11.7% on the upper extremities; and in 10% on the trunk. The incidence in the two sexes was nearly equal, there being 33 males and 27 females. There were 2 Negroes in the series. Of 25 patients presenting themselves in hospital practice comparatively late, only 2 were known to be alive after 5 years, whereas of 35 seen in dermatologic practice, 17 survived from 7 to 18 years. If the lymph nodes are involved the chances of cure by any method are practically nil.—H. G. W.

JAEGAR, J. R., and KINGRY, C. B. [Denver, Colo.] GLOMUS TUMOR. TUMOR OF THE NEUROMYO-ARTERIAL GLOMUS; SUBCUTANEOUS PAINFUL TUBERCLE. Rocky Mountain M. J., 38:717-720. 1941.

A case report with discussion.—R. C. R.

LAMB, J. H., GESCHICKTER, C. F., and LAIN, E. S. [Oklahoma City, Okla.] ANGIOMATOUS BASAL CELL TUMORS. South. M. J., 36:133-138. 1943.

Two cases of angiomatous basal cell tumors are added to Geschickter's series of 6 cases, none of which showed metastases. The tumors seem to arise from skin appendages and their relative malignancy may vary.—H. G. W.

ZIEMAN, S. A. [Chicago, Ill.] FIBROSARCOMA OF THE SOFT PART OF THE FINGER. Proc. Inst. Med. Chicago, 13: 284. 1941.

A case report.—R. C. R.

Nervous System

BOTSFORD, T. W. [Harvard Med. Sch., Boston, Mass.] PRIMARY BRAIN TUMOR. FOLLOW-UP STUDY OF 179 CASES. Am. J. Surg., 58:345-349. 1942.

An analysis of 179 cases of primary brain tumor seen at the Peter Bent Brigham Hospital between 1932 and 1941. The gross case mortality was 24.7% and the gross operative mortality was 16.3%. The majority of patients lived less than 2 years, although 72 survived from a few months to 8 years. Of the total series 59 (32.9%) are alive at the present time; 18 (30.5%) of the living patients are seriously crippled, while the remaining 41 (69.5%) are leading normal lives.—H. G. W.

ECHOLS, D. H. [Tulane Univ. School of Med., New Orleans, La.] LAMINECTOMY FOR SPINAL CORD TUMOR AND OTHER DISEASES. ANALYSIS OF 151 CONSECUTIVE CASES. New Orleans M. and Surg. J., 95:373-375. 1943.

An analysis of 151 cases emphasises the necessity for early diagnosis since spinal cord tumor is frequently a surgical emergency.—H. G. W.

GLOBUS, J. H., and KUHLENBECK, H. [Mt. Sinai Hosp., New York, N. Y.] TUMORS OF THE STRIATOTHALAMIC AND RELATED REGIONS. THEIR PROBABLE SOURCE OF ORIGIN AND MORE COMMON FORMS. Arch. Path., 34: 674-734. 1942.

Evidence is presented emphasizing the significance of embryonal and histogenetic factors in the production of brain tumors. It is demonstrated that these factors are operative particularly in the region designated as the striatothalamic junction, including the sulcus terminalis, and in other areas—the zone of coalescence of the anterior horns of the lateral ventricles and the subependymal cell plate about the nucleus caudatus and the septum pellucidum. These areas are frequent sites of tumor formation since they constitute common sites for embryonal residues. The spongioneuroblastic is the most common variety of tumor and is not infrequently multiple. Evidence is presented that the spongioneuroblastoma and its transitional forms are a type of tumor in which both neural and glial elements coexist in varying ratios. Tuberous sclerosis has frequently been found associated with the spongioblastic variety of tumor, and the fact that numerous ependymal granulations are frequently found in spongioneuroblastoma and tuberous sclerosis points to the significance of the subependymal plate as the source of neoplastic alterations.—H. G. W.

GLOBUS, J. H., and SAPIRSTEIN, M. [Mt. Sinai Hosp., New York, N. Y.] MASSIVE HEMORRHAGE INTO BRAIN TUMOR. ITS SIGNIFICANCE AND PROBABLE RELATIONSHIP TO RAPIDLY FATAL TERMINATION AND ANTECEDENT TRAUMA. J.A.M.A., 120:348-352. 1942.

A study of the significance of massive hemorrhage into brain tumors and its probable relationship to rapidly fatal termination was made in 94 cases of brain tumor not subjected to operation. The conclusion was drawn that

sudden death in the course of brain tumor is rarely associated with hemorrhage into the tumor or trauma to the head. Thrombosis is a much more important factor in sudden changes in the clinical course. Trauma to the head plays no part in the production of hemorrhage into a tumor.—H. G. W.

Breast

ADAIR, F. E. [Memorial Hosp., New York, N. Y.] THE ROLE OF SURGERY AND IRRADIATION IN CANCER OF THE BREAST. J.A.M.A., 121:553-559. 1943.

A study of 5 year end results in 3,535 cases, in which an attempt was made to evaluate surgery alone, preoperative irradiation, postoperative irradiation, and irradiation alone in the cure of operable breast cancer, revealed that the cases treated by surgery alone were a highly selected group and not representative of a cross section of the accomplishments of treatment by this method. The study shows that the preferable method of treating operable breast cancer is immediate radical mastectomy combined with postoperative irradiation. In the cases so treated, a 5 year survival was obtained in 76.8% of those with no axillary involvement, and in 41.8% of those with axillary involvement. The poorest end results observed in this study were in those unselected cases (24%) given irradiation only. The 5 year period was survived in 695 of the total 1,383 cases, making a salvage of 51%.—H. G. W.

BUDD, J. W. [Los Angeles Tumor Inst., Los Angeles, Calif.] PATHOLOGY OF THE BREAST. Radiology, 38:533-536. 1942.

The responses of breast tissue to irritation or stimulation are limited and are made up of various combinations of proliferation, functional differentiation, and involution. These reactions may be complicated by retrograde changes. The boundary between benign and malignant hyperplasia is arbitrary. The origin of sarcomas can usually be traced to old fibroadenomas. Epithelial proliferation may be considered benign only as long as the normal double layered epithelial cells remain in "symbiosis." Neoplasia may be arbitrarily defined as beginning in epithelial lesions when the two layers of differentiated cells cease to be formed and growth is of a uniform cell type. The earliest cancers are intraepithelial growths limited by intact basement membranes.—C. E. D.

ROSS, D. E. [Ross-Loos Medical Group, Los Angeles, Calif.] SURGICAL TREATMENT OF CARCINOMA OF THE BREAST. Am. J. Surg., 58:113-120. 1942.

A splendidly illustrated article giving the details of the operative removal of the breast.—H. G. W.

SCARFF, R. W., and SMITH, C. P. [Bland-Sutton Inst. of Pathology, Middlesex Hosp., London, England] PROLIFERATIVE AND OTHER LESIONS OF THE MALE BREAST. WITH NOTES ON 2 CASES OF PROLIFERATIVE MASTITIS IN STILBOESTROL WORKERS. Brit. J. Surg., 29:393-396. 1942.

The report is based on 65 specimens from partial or total mastectomy in the male examined at the Bland-Sutton Institute of Pathology during the years 1924-1940. Two of these are of particular interest in that they were from workers in stilbestrol, in each of whom hypertrophy of one breast occurred. In the whole series there were 19 malignant neoplasms, of which 15 were carcinomas arising in the gland tissue of the breast, 3 were sarcomas, and one was a rodent ulcer of the nipple. There were 5 benign neoplasms, and the remaining cases (41) were of chronic mastitis. Of the last, only 3 showed a "dangerous" degree of epithelial proliferation, and 2 of these were from the stilbestrol workers. Their ages were 18 and 23 and they had been handling stilbestrol or its precursors for 12 and 10 weeks respectively. The whole of the above material is analyzed and compared with similar lesions occurring in women during the same period. Chronic mastitis occurred in the male twice as commonly as cancer (the reverse proportion obtaining in women) but a degree of epithelial proliferation sufficiently advanced to give rise to apprehension of possible malignancy occurred in cases of chronic mastitis in men only half as frequently as in women.—A. H.

WORD, B., and REED, W. C. [U. S. Army Hosp., Camp Shelby, Miss.] BENIGN TUMORS OF THE MALE BREAST. Am. J. Surg., 59:106-112. 1943.

Twenty benign lesions of the male breast are reviewed and one unusual case of lipoma of the male breast is described in detail. Trauma was an etiological factor in but 2 of 14 cases.—H. G. W.

Female Genital Tract

BRINES, O. A., and BLAIN, J. H. [Alexander Blain Hosp., Detroit, Mich.] ADENOMYOSIS OF THE UTERUS. Surg., Gynec. & Obst., 76:197-203. 1943.

Adenomyosis is the result of spontaneous generation of endometrial stromal or interstitial cells from and within the myometrium. The stromal or interstitial cells thus produced differentiate later to form the gland cells of the endometrium. Adenomyosis of the uterus is definitely not a potentially malignant lesion.—H. G. W.

GRUENWALD, P. [Cook County Hosp., Chicago, Ill.] DEVELOPMENTAL BASIS OF REGENERATIVE AND PATHOLOGIC GROWTH IN THE UTERUS. Arch. Path., 35:53-65. 1943.

The formation of mesenchyme from the epithelium of the müllerian duct is described in detail. It occurs near the caudal end of the duct while the latter is growing toward the urogenital sinus. Similar formation of mesenchyme also takes place at the expense of the nearby peritoneal epithelium, the so called tubal ridge. Consequently, the nonepithelial tissues of the uterovaginal canal arise not only from the mesenchyme originally present in that area but also, in part, from the epithelia of the inner and outer linings of the canal. Thus a strong possibility exists that these nonepithelial cells may possess the developmental potencies of their epithelial coverings. These facts make reports of postmenstrual regeneration of uterine epithelium from the stroma appear less improbable than is generally assumed. Furthermore, the possibility must be taken into account that a clear cut distinction of epithelial and nonepithelial structure may not always be possible in tumors of derivatives of the celomic wall.—H. G. W.

HARTZ, P. H., and HUGENHOLTZ, M. J. [Public Health Service, Curaçao, N.W.I.] MITOTIC ACTIVITY IN UTERINE LEIOMYOMAS. Am. J. Clin. Path., 12:523-524. 1942.

In contrast to the view that mitotic division is seldom or never seen in uterine leiomyomas, the authors, by using

an improved technic, found mitotic figures in 63% of these tumors, and in 21% found them easily and in great numbers.—H. G. W.

TEAHAN, R. W., and WAMMOCK, H. [Philadelphia, Pa.] CARCINOMA OF THE VULVA. Pennsylvania M. J., 44:1268-1274. 1941.

The authors feel strongly that a prompt diagnosis and adequate early treatment will bring about a pronounced increase in the cures of this disease. Radiation very seldom cures carcinoma of the vulva and frequently delays the institution of proper treatment to such an extent that cure becomes impossible. Radical surgery should be carried out whenever feasible.—J. L. M.

Male Genital Tract

ALYEA, E. P., and HENDERSON, A. F. [Duke Univ. Sch. of Med., Durham, N. C.] CASTRATION FOR CARCINOMA OF THE PROSTATE: A REPORT OF THE IMMEDIATE RESULTS. J. Urol., 48:673-681. 1942.

A report of a series of 40 cases in which immediate general improvement and relief of metastatic pain was obtained. Changes in the primary growth following castration were evident clinically and at autopsy; x-rays showed the healing of bone in metastatic areas and disappearance of metastatic tumors in the lungs. Stilbestrol caused regression of the primary growth and clinical improvement but not to the same extent as castration.—H. G. W.

CHUTE, R., WILLETTS, A. T., and GENS, J. P. [Massachusetts General Hosp., Boston, Mass.] EXPERIENCES IN THE TREATMENT OF CARCINOMA OF THE PROSTATE WITH STILBESTEROL AND WITH CASTRATION BY THE TECHNIC OF INTRA-CAPSULAR ORCHIDECTOMY. J. Urol., 48:682-692. 1942.

Reduction of the action of androgens in the body, either by surgical castration, or by biochemical neutralization by the administration of the synthetic estrogen stilbestrol, or by a combination of the two, the method used in a majority of the cases (77%), benefited 26 out of 27 cases of inoperable carcinoma of the prostate. No beneficial effects on bone metastases were noted. The acid phosphatase, if elevated, fell rapidly toward normal following castration or stilbestrol therapy, whereas the alkaline phosphatase usually rose. Estimations of the 17-ketosteroids were made in 18 cases before and after castration and the impression was gained that the level of the 17-ketosteroids does not give information of value.—H. G. W.

DEAN, A. L., WOODARD, H. Q., and TWOMBLY, G. H. [Memorial Hosp., New York, N. Y.] ENDOCRINE TREATMENT OF CANCERS OF THE PROSTATE. J. Urol., 49:108-117. 1943.

Although patients with prostatic cancer are more comfortable after castration or the administration of estrogens, the majority die, but the duration of life may be somewhat longer. The clinical benefit that follows the oral administration of stilbestrol seems to be as great as that following castration. It seems well demonstrated that the natural history of cancer of the prostate may be definitely modified by changes in the endocrines. This idea possibly gives a starting point for the cure and also the prevention of prostatic cancers.—H. G. W.

FETTER, T. R. [Jefferson Med. Coll., Philadelphia, Pa.] THE MANAGEMENT OF TUMOR OF THE TESTICLE. A STUDY OF 42 CASES. Pennsylvania M. J., 44:1240-1250. 1941.

An attempt is made to point out the proper management of tumor of the testicle by emphasizing the need for early recognition of the tumor, early orchiectomy, the role of roentgen irradiation, the significance of hormone studies in correlation with the histologic picture, and subsequent treatment including a regular physical, roentgenologic, and hormone check up for metastases. Forty-two cases of tumor of the testicle are reported. Fifteen patients are living, the survival time being from 4 months to 12 years. Five of the living patients are not well, 2 have demonstrable metastases. Twenty-seven patients are dead, 7 died within the first year of treatment, 5 of the 27 had evidence of metastases. Eighteen lived for 1 to 6 years, 1 for 8 years, and 1 survived 10 years.—J. L. M.

GILBERT, J. B. [Albany, N. Y.] STUDIES IN MALIGNANT TESTIS TUMORS. VIII. TUMORS IN PSEUDOHERMAPHRODITES: REVIEW OF 60 CASES AND A CASE REPORT. J. Urol., 48:665-672. 1942.

A report is made of an external female pseudohermaphrodite with embryonal carcinoma of an abdominal testicle, and 60 cases of tumors in pseudohermaphrodites, found in the literature, are reviewed.—H. G. W.

RANDALL, A. [Philadelphia, Pa.] EIGHT-YEAR RESULTS OF CASTRATION FOR CANCER OF THE PROSTATE. J. Urol., 48:706-709. 1942.

Among 5 patients with carcinoma of the prostate, castrated 7 to 9 years ago, none was cured, although relief was obtained in some cases.—H. G. W.

ROSENBLATT, P., GRAYZEL, D. M., and LEDERER, M. [Jewish Hosp., Brooklyn, N. Y.] PRIMARY MALIGNANT TUMORS OF THE TESTICLE. Am. J. Surg., 57:94-103. 1942.

An analysis of 29 tumors, 20 of which were homologous and 9 heterologous. In 10% there was a history of trauma. The heterologous tumors appeared in an earlier age group and offer a poorer prognosis than the homologous tumors.—H. G. W.

Urinary System—Male and Female

BARRINGER, B. S. [Memorial Hosp., New York, N. Y.] THE IMPONDERABLES IN GENITOURINARY CARCINOMA. Am. J. Surg., 58:404-406. 1942.

A report of 3 patients, apparently dying of genitourinary carcinoma, who were given intraspinal alcohol injections to relieve pain and have unexpectedly survived, 2 of them for 5 years.—H. G. W.

BOTHE, A. E. [U. S. Naval Hosp., Bethesda, Md.] CARCINOMA OF THE RENAL PELVIS AND URETER. J. Urol., 49:69-76. 1943.

Pathological studies of 6 cases of carcinoma of the renal pelvis and ureter show that the early changes that first involve the subepithelial supporting tissue are inflammatory in type. This is followed by proliferation of the basal layer which eventually becomes papillary or infiltrating.—H. G. W.

KIRWIN, T. J. [New York Hosp., New York, N. Y.] PAPILLOMATOSIS OF THE BLADDER: NEW CONCEPTIONS OF ETIOLOGY AND TREATMENT. J. Urol., 49:1-13. 1943.

On the basis of the hypothesis that vesical papillomatosis is the result of a virus, the author recommends excision

of the growths with the wire loop electrode, cauterization of the bases with the ball electrode, and sterilization of the bladder mucosa with phenol.—H. G. W.

KREUTZMANN, H. A. R. [Mt. Zion Hosp., San Francisco, Calif.] PRIMARY LYMPHOSARCOMA OF THE BLADDER. J. Urol., 48:147-152. 1942.

A report of a fifth case of lymphosarcoma of the bladder. The tumor apparently originates from lymphoid tissue formed in response to chronic irritation and is a localized condition without evidence of generalized glandular involvement.—H. G. W.

LIVERMORE, G. R. [Memphis, Tenn.] CARCINOMA OF THE BLADDER. J. Urol., 49:164-170. 1943.

A plea for early radical treatment of carcinoma of the bladder.—H. G. W.

MITCHELL, N., and ANGRIST, A. [Queens General Hosp., Jamaica, N. Y.] ADRENAL RESTS IN THE KIDNEY. Arch. Path., 35:46-52. 1943.

In 1,806 necropsies, 22 adrenal rests in the kidneys were disclosed.—H. G. W.

Oral Cavity and Upper Respiratory Tract

WATSON, W. L. [Memorial Hosp., New York, N. Y.] CANCER OF THE TRACHEA. FIFTEEN YEARS AFTER TREATMENT FOR CANCER OF LARYNX. J. Thoracic Surg., 12:142-150. 1942.

The tracheal carcinoma arose at the point of irritation by the tracheotomy tube that had been inserted 15 years before at the time of operation for cancer of the larynx.—H. G. W.

WOOKEY, H. [Univ. of Toronto, Toronto, Canada] THE SURGICAL TREATMENT OF CARCINOMA OF THE PHARYNX AND UPPER ESOPHAGUS. Surg., Gynec. & Obst., 75:499-506. 1942.

Methods are described for removing operable growths involving the lower pharynx and upper esophagus.—H. G. W.

Salivary Glands

McFARLAND, J. [Univ. of Pennsylvania Med. Sch., Philadelphia, Pa.] THE MYSTERIOUS MIXED TUMORS OF THE SALIVARY GLANDS. Surg., Gynec. & Obst., 76:23-34. 1943.

The mixed tumors of the salivary glands are a group by themselves. It is impossible to be dogmatic about them for they disregard every canon of oncology and continually do the most unexpected things. It is impossible through the microscopic study of their structure to foretell what any of them will do, and any variety may recur. The long delay before recurrence deludes the surgeon and roentgenologist into believing that he has been successful in his treatment, when no good at all has resulted. The same long delay and the occasional recurrence of the tumor 20, 30, or 40 years after operation justifies the question, "Is any patient, once the victim of such a tumor, ever cured?" —Author's summary.

PERRIN, T. L. [Nat. Inst. of Health, U. S. Public Health Service, Washington, D. C.] MIXED TUMOR OF THE PAROTID WITH METASTASES. Arch. Path., 33:930-934. 1942.

A report of a case of mixed tumor of the parotid. Distant metastases exhibited the histologic structure of the parent tumor.—H. G. W.

SHELDON, W. H. [Harvard Med. Sch., Boston, Mass.] SO-CALLED MIXED TUMORS OF THE SALIVARY GLANDS. Arch. Path., 35:1-20. 1943.

The so called mixed tumors of the salivary glands include cancerous and noncancerous neoplasms composed of one or two different types of neoplastic cells. Both types of cells are normally present in the salivary glands, one represented by the secreting epithelium, the other by the basket cells. The latter are peculiar smooth muscle cells belonging to the myoepithelium. Some of these neoplasms arise from the epithelium. In these tumors an excessive and probably also qualitatively abnormal secretion produces a peculiar myxomatous and pseudocartilaginous appearance of the connective stroma. True cartilage may be present and is formed by metaplasia from the stroma. Other neoplasms arise from both the epithelium and the basket cells. These are truly organoid tumors which closely reproduce the normal components and structure of the salivary glands. Squamous metaplasia of the epithelium, myxomatous and pseudocartilaginous stroma, and true cartilage and bone may be present. A few neoplasms arise from the basket cells alone. The presence of myoepithelial cells in these neoplasms accounts for the resemblance to tumors of the sweat glands, the mammary glands, and probably also the lacrimal and ceruminous glands.—H. G. W.

WILSON, H. [Univ. of Tennessee Med. Sch., Memphis, Tenn.] MASSIVE MIXED TUMOR OF THE SUBMAXILLARY GLAND. Am. J. Surg., 58:426-428. 1942.

A report of a tumor, 13 by 9 cm., removed under local anesthesia.—H. G. W.

Intrathoracic Tumors—Lungs—Pleura

FREEDLANDER, S. O., and GREENFIELD, J. [Western Reserve Univ., Cleveland, Ohio] HEMOPTYSIS IN METASTATIC TUMORS OF THE LUNG SIMULATING BRONCHIOGENIC CARCINOMA. J. Thoracic Surg., 12:109-116. 1942.

Two cases are reported of metastatic tumor invasion of bronchi with the presenting symptom of hemoptysis simulating primary bronchiogenic carcinoma.—H. G. W.

HARRIS, W. H., Jr. [Tulane Univ. Sch. of Med., New Orleans, La.] HISTOLOGIC ANALOGY OF BRONCHIAL ADENOMA TO LATE PRENATAL AND EARLY POSTNATAL STRUCTURES. Arch. Path., 35:85-92. 1943.

The observations reported here point out the similarity of infantile types of structures, especially the bronchial mucous glands and the peribronchial and peritracheal lymphadenoid tissue, to the histologic findings in bronchial adenoma.—H. G. W.

STOUT, A. P., and MURRAY, M. R. [Presbyterian Hosp., New York, N. Y.] LOCALIZED PLEURAL MESOTHELIOMA. INVESTIGATION OF ITS CHARACTERISTICS AND HISTOGENESIS BY THE METHOD OF TISSUE CULTURE. Arch. Path., 34:951-964. 1942.

An investigation of a pleural tumor by the method of tissue culture leads to the conclusion that the tumor is truly a mesothelioma. In a footnote, however, Pappenheimer throws doubt on this conclusion, and suggests that the growth is really a secondary tumor from an apparently benign uterine myoma.—H. G. W.

Gastrointestinal Tract

ABRAHAMSON, R. H., and HINTON, J. W. [Bellevue Hosp., New York, N. Y.] THE GASTRIC MUCOSA AS AN ENDOCRINE GLAND. Surg., Gynec. & Obst., 76:147-163. 1943.

The conception is presented that the gastric mucosa can act as an endocrine gland by means of hormones. There are pronounced differences in blood hormone content between the sexes, which may explain the sex discrepancy in gastric cancer.—H. G. W.

GARLOCK, J. H., GINZBURG, L., and GLASS A. [Mt. Sinai Hosp., New York, N. Y.] COMPLICATIONS AND CAUSES OF MORTALITY OF THE SURGICAL TREATMENT OF CARCINOMA OF THE COLON AND RECTUM. Surg., Gynec. & Obst., 76:51-59. 1943.

A discussion of operative technic.—H. G. W.

KIRKLIN, B. R., and MacCARTY, W. C., Jr. [Mayo Clinic, Rochester, Minn.] INCIDENCE OF MALIGNANCY IN PREPYLORIC ULCERS. J.A.M.A., 120:733-735. 1942.

The prevalent assumption that prepyloric ulcers are more likely to be malignant than ulcers situated elsewhere in the stomach has been challenged in recent years. To obtain information that might help to solve the question, ulcerous prepyloric lesions observed roentgenologically and operated on at the Mayo Clinic during the period 1937 to 1941 inclusive were reviewed. All ulcerating carcinomas that had been diagnosed roentgenologically were excluded from consideration. After other exclusions made necessary by the roentgenologic approach, there remained 61 ulcerating carcinomas and 71 ulcers; of the latter, 63 were benign and 8 (11.3%) malignant. These figures are compatible, though not identical, with the generally accepted estimate that from 10 to 12% of all gastric ulcers prove to be malignant. This study thus supports the newer view that prepyloric ulcers are not more often carcinomatous than are gastric ulcers in other locations.—H. G. W.

PICKWORTH, M. E. [San Jose, Calif.] MULTIPLE ADENOMATOSIS OF THE COLON. CASE REPORTS. Am. J. Surg., 58:254-257. 1942.

Three cases of multiple adenomatosis of the colon are presented, in all of which malignancy had developed. One patient had a family history of bowel malignancy.—H. G. W.

SAPHIR, O., and PARKER, M. L. [Michael Reese Hosp., Chicago, Ill.] LINITIS PLASTICA TYPE OF CARCINOMA. Surg., Gynec. & Obst., 76:206-213. 1943.

Of the 30 cases of linitis plastica observed, 26 involved the stomach, 3 the large intestine, and 1 the gall bladder. The time interval between the onset of symptoms and death was much shorter than with other types of cancer and the survival time after operation was very brief. Metastasis to the liver was found only 3 times, but among 6 females the ovaries were involved 3 times. It is very questionable whether linitis plastica, in the sense of a purely inflammatory, nonmalignant lesion, exists.—H. G. W.

SATORY, J. J. [Milwaukee County Hosp., Wauwatosa, Wis.] MALIGNANT CARCINOID TUMORS OF THE GASTROINTESTINAL TRACT. Am. J. Surg., 58:275-278. 1942.

A report of a carcinoid tumor growing from the stump of the appendix into the cecum with metastases to the regional lymph nodes. This is the 77th reported case of malignant carcinoid tumor.—H. G. W.

SMITH, T. E. [Baylor Univ. Coll. of Med., Dallas, Texas] PRIMARY LYMPHOID TUMORS OF THE RECTUM RESEMBLING INTERNAL HEMORRHOIDS. REPORT OF THREE CASES. J.A.M.A., 121:495-497. 1943.

The 3 cases here reported are the only ones to be found in the literature.—H. G. W.

STONE, H. B., and McLANAHAN, S. [Baltimore, Md.] RESECTION AND IMMEDIATE ASEPTIC ANASTOMOSIS FOR CARCINOMA OF THE COLON. J.A.M.A., 120:1362-1365. 1942.

Based on an experience of 147 cases, the conclusion is reached that, in carcinoma of the colon, resection and aseptic anastomosis is the ideal operative procedure.—H. G. W.

Bone and Bone Marrow

LEVITT, A., and LEVY, D. S. [Buffalo City Hosp., Buffalo, N. Y.] MULTIPLE MYELOMA. Ann. Int. Med., 17:863-873. 1942.

This case of a 25 year old male was remarkable for the total absence of Bence-Jones proteinuria and for an absorption of bone so extensive that the body of the patient seemed like a flattened, collapsed, fluctuant mass.—H. G. W.

SPEED, K. [Presbyterian Hosp., Chicago, Ill.] POST-METASTATIC SURVIVAL OF OSTEOGENIC SARCOMA. Surg., Gynec. & Obst., 76:139-146. 1943.

A report of the survival for 13 years of a patient with an osteogenic sarcoma of the femur and numerous pulmonary metastases.—H. G. W.

Blood Vessels

BAILEY, W., and KISKADDEN, W. S. [Los Angeles, Calif.] TREATMENT OF HEMANGIOMATA, WITH SPECIAL REFERENCE TO UNSATISFACTORY RESULTS. Radiology, 38:552-561. 1942.

Success in the treatment of hemangiomas often depends on selection of the appropriate form of therapy. Radiation usually gives poor results in capillary hemangiomas or "port wine stain" since doses large enough to produce permanent skin injury are necessary to destroy the mature capillary endothelium. The hypertrophic endothelial hemangioma or "strawberry mark" is made up of masses of proliferating endothelium and generally responds well to radiation. Surgical excision is usually the treatment of choice in cavernous hemangioma, although radiation may sometimes be used. Sclerosing solutions, carbon dioxide snow, and electrocoagulation have been successfully used but have several disadvantages. The technic of surface and interstitial radium and radon application is discussed in detail.—C. E. D.

Correction

The author of the paper entitled "The Distribution of Doses of Radioactive Phosphorus in Leukemic Patients," Cancer Research, **3**:334-336. 1943, wishes it said that mc. in lines 5, 11, and 12 on page 336 should have been μc.